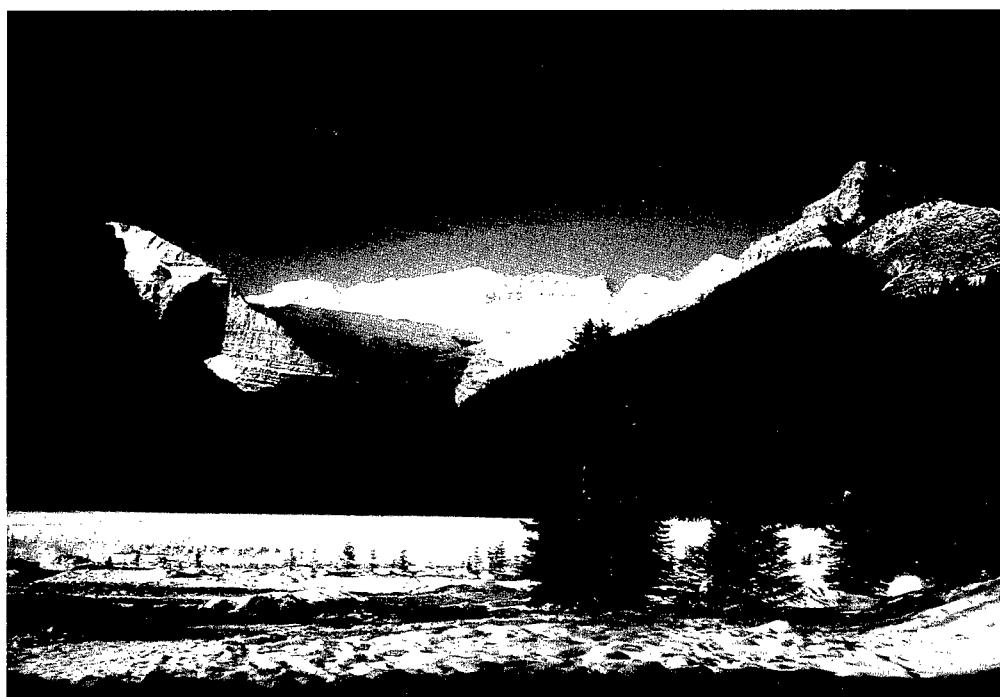


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HYPOXIA
WOMEN AT ALTITUDE

HYPOXIA

WOMEN AT ALTITUDE

**PROCEEDINGS OF THE TENTH INTERNATIONAL HYPOXIA SYMPOSIUM AT LAKE
LOUISE, CANADA, FEBRUARY 18-22, 1997.**

EDITORS:

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HYPOTENSION

WOMEN AT ALTITUDE

EDITORS: CHARLES S. HOUSTON AND GEOFFREY COATES

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This year as always the Management and staff of the Chateau Lake Louise were exceptionally helpful and a delight to work with. Stefan Deprez the director of convention and conference services deserves our special thanks.

The financing of these symposia has always been problematic. This year we are grateful to The US Army Medical Research Acquisition Activity and to the Medical College of South Carolina for their generous financial support.

This was our final year as organizers of these symposia. We leave with a mixture of sadness and joy. The sadness comes with any "ending" the joy comes from the many friendships that we have developed at these symposia over the years. The leadership of The Hypoxia Symposia is now in the capable hands of Peter Hacket and Robert Roach. With their leadership the 11th International Hypoxia Symposium promises to be as exciting as ever. Please join us for the full moon at Lake Louise in February 1999.

Geoff Coates
Charles Houston
Co-Chairs, Hypoxia 1997

IN MEMORIAM



John Sutton
1941-1996

John died in his sleep at home in Sydney on February 7th 1996. "For everything there is a season" but it is hard to accept that someone so vital, so bursting with energy and imagination so filled with ideas and hopes and plans should leave us. Meeting John was like meeting a six foot high tornado, he was bigger than life and seemed invulnerable. But he wasn't.

The first event at the 1997 Hypoxia Symposium was a celebration of the life of this remarkable human being John Sutton. He was a scientist, an explorer, a physician, an athlete and a friend to many of us, but above all he was a husband and father whose family meant the world to him.

John put much of himself into Hypoxia for the past twenty years. He was the energy behind these Symposia and they will never be quite the same without him. As a tribute to John a special memorial fund has been established in his memory. All who loved and admired him are invited to contribute.*

Geoff Coates
Charles Houston

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DEDICATION

**Robert F. Grover MD, PhD
High Minded Medical Scientist**

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Robert F. Grover, M.D., Ph.D.: High-Minded Medical Scientist

Bob hasn't always been a scientist; but his inquiries into the natural world began in childhood, with nearly disastrous chemistry experiments at home in his basement.¹ In 1940, when he entered Cornell University at age 16, Bob did his first experiment in what we now call integrative physiology and pharmacology. He caused a 'borrowed' cat to sleep for days in his college room when he gave it an hypnotic agent to test the effects. He began his love of mountains during World War II when he was a radioman in a Signal Corps station atop the Zugspitz in the Alps. Bob's interest in

physiology began on a troop ship, where close quarters and plenty of time for conversation, allowed a fellow soldier with a PhD, to tell him about careers in physiology. After the war, and after graduating in chemistry from University of Rochester in 1947, Bob began his PhD at the University of Colorado Medical School, Denver, where he combined the study of physiology with a proximity to the mountains. Bob's wife, Estelle, ever a constant friend and companion throughout his career, worked to support both of them through his doctoral programs, and subsequently collaborated in his research studies.

By 1948 Bob had attracted the attention of the Chief of Surgery, Dr. Henry Swan, who wanted to measure intra-operative aortic and pulmonary arterial pressures before and after ligation of the ductus arteriosus. Bob hid under drapes in the operating room to record the pressures,² which followed by only 3 years the first measurement of pulmonary arterial pressure in man. The results not only showed for the first time the physiology of high flow through the ductus arteriosus, but also a correctable form of pulmonary hypertension. Bob got his PhD degree in 1951, but was soon convinced by Dr. S. Gilbert Blount, Chief of Cardiology, to get his MD degree, and then to run the young cardiac catheterization laboratory at the medical school.

In 1957, during his first year in the catheterization laboratory, Bob was inexorably drawn into high altitude research. Drs. Arch Alexander and Don Will at Colorado State University School of Veterinary Medicine at Fort Collins had found pulmonary hypertension in cattle at high altitude, and needed help recording the

pressures. Thus began the continuing collaboration between the medical and veterinary schools and also Bob's career long study of life at high altitude. From 1957 on it was clear that the hypoxia of high altitude not only affected the lung circulation, but also many facets of oxygen transport. High altitude induced changes in the lung circulation and in body-wide oxygen transport then became the twin paths Bob has followed ever since.

In the subsequent decades, work which Bob did, or which was done under his direction as head of the Cardiovascular Pulmonary Research Laboratory has been truly remarkable, a few of the published articles are briefly detailed below:

* Reported that at 1600m pulmonary vasodilation was possible in children with left to right shunts.³

* Found that cattle, but not sheep develop severe pulmonary hypertension over several weeks at 12,700 feet altitude. There is remarkable variability in the pulmonary hypertensive response both between and within species.^{4,5,6,7,8}

* Showed (with Vogel) that the newborn was particularly susceptible to hypoxic pulmonary hypertension.⁹

* Reported that severe, but reversible pulmonary hypertension was present in healthy school children in Leadville, CO at 10,150 feet.^{10,12}

* Was the first to show that basal oxygen uptake increases at high altitude.¹¹

* Showed that athletes born and raised at 10,150 feet were not better track competitors than athletes from sea level, either at sea level or at altitude.¹³

* Was the first to report that cardiac output and stroke volumes were reduced at moderate altitudes of 3000 m.^{14,15}

* Reported (with Weil) that red cell mass has a curvilinear response to altitude, which resembles that of ventilation to altitude.¹⁶

* Showed (with Weil) that decades-long residence at altitude attenuated chemoreceptor function.¹⁷

* Showed (with Hultgren) that subjects susceptible to high altitude pulmonary edema, who were re-exposed to high altitude, rapidly developed an excessive pulmonary hypertension not immediately reversed by oxygen.¹⁸

* Examined (with Dempsey) effect of altitude residence on gas exchange.¹⁹

* Was the first to show that supplemental CO₂ at high altitude maintained exercise stroke volume, but impaired arterial oxygenation.²⁰

* Described (with Scoggin) re-entry high altitude pulmonary edema in children of Leadville, CO.²¹

* Described (with Will) the additive interaction of cold and hypoxia on pulmonary hypertension in cattle.²²

* Described (with Hackett) the association of high altitude pulmonary edema and congenital absence of right pulmonary artery in humans.²³

* Suggested (with Kryger) that chronic mountain sickness excessive polycythemia might be related to poor oxygenation during sleep at altitude.²⁴

Although, Bob's studies at altitude have encompassed nearly every organ system, a great variety of animal species, an extended range of altitudes, and individuals with a wide range of ages, in altitude chambers and in the field, his influence is not limited to his published work. At the University of Colorado, he established and led for a quarter century the Cardiovascular Pulmonary Research Laboratory which became one of the world's leading scientific groups for hypoxic research. Dozens of basic and clinical scientists who were trained there went on to distinguished inde-

pendent careers. He showed them by example, the art of investigation, the importance of collaboration and the joy of inquiry.

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CHAPTER 1

WOMEN AT ALTITUDE

OVERVIEW

by
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University of Colorado Health Sciences Center
² Department of Anthropology, University of Colorado at Denver

Introduction

To date there has been little systematic study of women's physiological responses to acute or chronic hypoxia. Despite the early, inclusive studies conducted on residents of high altitude by Mabel Purefoy Fitzgerald,¹⁰ most of the extensive literature on newcomers or long-term residents of high altitude has not included female subjects. Such an omission is consistent with much of the cardiovascular, respiratory and exercise physiology literature. One suspects that it is due, in part, to a concern that cyclic variation in female hormones may contribute an additional source of variation. Ironically, this very possibility is actively being investigated in contemporary studies of women at altitude.

As detailed in the papers and abstracts of this symposium, influences of cyclic variation in ovarian hormones during the menstrual cycle and comparisons between women and men are currently underway concerning the effects of high altitude on ventilatory acclimatization, pulmonary diffusing capacity, blood volume regulation, cardiac and blood pressure regulation, energy requirements, substrate utilization, and symptoms of acute mountain sickness. Such studies are prompted by the previous lack of attention paid to the female half of the human population and the recognition that sex-related hormones may influence the physiological responses observed.^{29,35}

Sex Hormone Influences on Oxygen Transport

Ovarian hormones fluctuate during the menstrual cycle (Fig. 1). During the follicular phase, estradiol levels gradually rise. A surge in luteinizing hormone prompts the release of the egg. Once released, the follicle becomes the corpus luteum and secretes progesterone. If implantation does not occur, the uterine lining (decidua) is shed, menses ensues, and the next follicular period begins.

The luteal phase is remarkable in several respects. Overall, it may be viewed as a preparation for pregnancy; the increased blood volume, cardiac output, and ventilation presage and may be important for sustaining the early days of pregnancy.⁷ As a regularly occurring event, the luteal phase is uniquely human. It is more frequent today in industrialized countries than 100 years ago or in other regions of the world as the result of a younger age of menarche, decreased number of pregnancies, reduction in the duration of lactational amenorrhea, and advancing age of menopause.

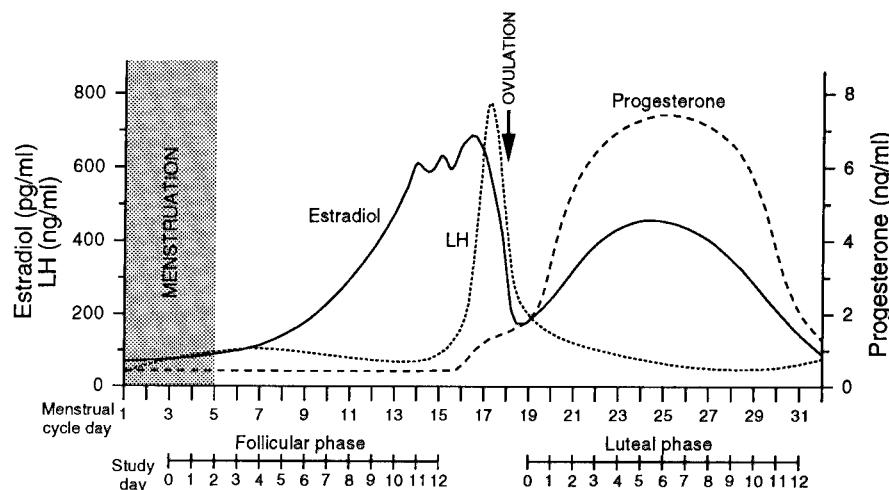


Figure 1 Hormonal fluctuations of the normal, human menstrual cycle.

The hormonal fluctuations of the menstrual cycle influence ventilation.^{1,29} Progesterone raises resting ventilation and, together with estrogen, acts on carotid body and central nervous system sites to raise hypoxic ventilatory response.¹⁴ This increase in hypoxic ventilatory response is most apparent during the luteal phase when end-tidal PCO_2 is restored to follicular values. Because the hypoxic drive to breathe at sea level determines in large part ventilation at altitude,²⁵ the study reported by Muza and co-workers in this volume tested the hypothesis that women acclimatize faster during the luteal than follicular phases of the menstrual cycle. Although the higher arterial O_2 tensions of the luteal phase have little effect on O_2 content or maximal exercise performance at sea level, the improved oxygenation of the luteal phase at high altitude would be expected to increase arterial O_2 saturation which, in turn, might widen the peripheral diffusion gradient for O_2 delivery and improve exercise performance.

The hormonal fluctuations of the menstrual cycle have major cardiovascular effects. In the luteal phase when both estradiol and progesterone are elevated, cardiac output, stroke volume and plasma volume tend to be greater than in the follicular phase.⁷ This may be due to vasodilatory effects of estradiol and progesterone on both veins and arterioles as well as to their stimulatory effects on plasma renin and aldosterone secretion which, in turn, act to retain salt and water. The vasodilatory actions of estradiol are chiefly mediated by activation of endothelial alpha 2-adrenergic receptors^{21,31} which, in turn, increase endothelial production of nitric oxide^{13,30} and vasodilator prostaglandins.³² Estradiol activation of vascular smooth muscle of alpha 2-adrenergic receptors in endothelium-denuded vessels decreases the contractile response to norepinephrine in the presence of inhibitors of neuronal uptake, extraneuronal uptake and beta-receptors,³ suggesting that estradiol decreases vascular smooth muscle sensitivity to norepinephrine. However, estradiol also has alpha 1-adrenergically mediated vasoconstrictor effects; estradiol increases the number and affinity of alpha 1-adrenergic receptors to raise contractile response to norepinephrine.^{4,8} Progesterone is also known to relax venous smooth muscle²⁰ and generally oppose alpha 2-adrenergic mediated vasorelaxation.²¹

Sympathetic stimulation occurs in response to high altitude in men.²⁴ Women have similar heart rate increases but the contribution of sympathetic stimulation is unknown.⁹ In men at altitude, cardiac output increases on arrival and then falls below sea level values by 5 days.²⁴ While differences between the phases of the menstrual cycle are to be expected from the low-altitude data, the changes in cardiac output that occur in women at high altitude are unknown. In men at altitude, venous tone increases and plasma volume decreases.^{24,34} Limited data in women suggest that plasma volume shrinkage is reduced both in rate and magnitude.¹⁶

Metabolic rate increases with altitude exposure in men, requiring increased calories to prevent weight loss.⁵ Gender differences in the effect of altitude on metabolic rate are supported by the shorter duration of hypophagia and lesser weight loss observed in women than men.^{9,17,36} In men, when calories were sufficient under conditions of hypoxia, glucose appeared to be preferred over fat for fuel.^{5,26} Because the increase in glucose utilization correlated with norepinephrine concentrations, it appeared as though the altitude-associated increase in glucose utilization was under alpha-adrenergic control. The preferred metabolic fuel for women at altitude is neither known nor easily predicted. At sea level, women utilize fat more than men, especially during the luteal phase. Estradiol-related actions include: 1) depression of gluconeogenesis and glycogenolysis,² 2) an increased insulin:glucagon ratio which favors lipid utilization,¹⁹ and 3) increased circulating growth hormone which promotes fat utilization.¹² Thus, the hormonal influences present in the luteal phase all favor metabolism of fat over glucose. If this pattern is preserved in women at high altitude, an increased dependence on fat would be opposite to the increased dependence on glucose observed in men. A greater lipolytic activity in women at sea level may be the cause of their greater endurance capacity for a given percent VO_2 max than men.¹¹

Hemoglobin concentrations in healthy women living permanently at sea level and at high altitude are about 11% less than those of men but show greater variability in response to altitude.¹⁰ Progesterone inhibits the stimulatory actions of testosterone on erythropoietin production²² and estradiol exerts direct inhibitory effects on erythropoietin.²³

Thus, ovarian hormones influence many of the ventilatory, cardiovascular, metabolic, volume regulatory and hematological acclimatization responses to high altitude. Their influence on acclimatization has not been systematically studied in the past. It is therefore the subject of this symposium and reports presented elsewhere at this Hypoxia Symposium.

Important Conceptual Caveats

It is tempting to equate sex differences with the presence or absence of ovarian hormones but there are several reasons why this is incorrect or at best naive.

1) Men have hormones too. Ovarian hormones characterize women and fluctuate during the menstrual cycle but are not the only sex hormones with physiological effects. Testosterone has marked effects on oxygen transport-related processes^{28,29} and undergoes fluctuations over time.

2) The determination of sex involves a complex combination of genetic, prenatal, and developmental factors. In mammals, sex is determined by having received from one's parents either two X chromosomes (females) or one X and one Y chromosome (males) but this chromosomal determination of sex is not universal. Among birds, males are XX and females are XY. Sex determination in most insects is the

same as in mammals but in two orders, the lepidoptera (moths and butterflies) and trichoptera (caddisflies), sex determination is like that of birds (XX males and XY females).¹⁸ Developmental factors are also involved in the determination of secondary sexual characteristics. The expression of a gene or genes on the Y chromosome together with a brief period of elevation in androgen levels during embryonic life is required to prompt the formation of testes and become phenotypically male. If this gene(s) on the Y chromosome is lacking, an XY individual will develop into a female; likewise a prenatal surge in androgens can cause a genetic female to develop testes. Since, in the absence of a stimulus to become male, a female will result, female features appear to be the underlying or primary attribute of mammalian life.

3) Sex-specific genetic material is transmitted from parent to offspring. Mother and father contribute an equal, haploid number of chromosomes to form the zygote but, in addition, in humans as well as other animals, the mother contributes her mitochondrial DNA to the fertilized egg. This mitochondrial DNA reproduces itself with each ensuing cell division. In a somewhat analogous fashion, Y-chromosomal DNA is transmitted only by the father. However an important difference is that Y-chromosomal DNA is acquired only by males whereas both females and males possess the maternally-derived mitochondrial DNA. Thus the developing organism is subject to nuclear genetic influences from both parents but to mitochondrial genetic influences from its mother only.

4) There are additional means by which maternally and paternally-derived genetic material can influence the developing organism; namely, imprinting or the disproportionate influence of maternal or paternal genetic material. Recent studies have revealed that, for example, paternal genes exert a greater influence on placentation whereas maternal genes appear to predominate in fetal development.¹⁵

5) Time-dependent effects influence the developing organism. That is, during embryonic, fetal as well as postnatal life, the maturation and functioning of sex-related characteristics affects the progression of events at subsequent ages. For example, sex differences in ventilation and ventilatory sensitivity to hypoxia are not fully reversed by the removal of testes and ovaries in cats.²⁷ Likewise in sheep, gonadectomy does not completely eliminate sex differences in pulmonary vasoreactivity.³³ It is not clear whether the retention of sex differences in these studies was the result of other, sex chromosome-linked factors or to the later-in-life consequences of earlier events triggered by circulating testicular or ovarian hormones.

6) As mentioned above, the human menstrual cycle is unique insofar as no other mammal (including other primates) has a menstrual cycle. Other mammals have estrous cycles which are not the same or even very similar to the menstrual cycle. In an estrous cycle, the visible manifestations of estrous mark the time of ovulation which, in the human case, typically occurs after the completion of menstruation between the follicular and luteal phases. In other animals, estrous is an infrequent occurrence, taking place once or perhaps several times a year but not as often as once each month. The relationship between the phases of the menstrual cycle, mating, and estrous is also distinctive. Humans are anestrous, meaning that mating bears no particular relationship to the time of estrous. All other mammals mate only at times of estrous or "heat". The beginning of this period is similar to the onset of the follicular phase insofar as estrogen levels are rising. Unlike humans, however, there are obvious, visible, behavioral, and odor signals marking the time of estrous. The act of mating is directly related and, in fact, required in many species for the maturation

and release of the egg. The stimulus of estrous is sufficiently strong that mating is effectively guaranteed. Once mating has occurred and conception successful, the familiar events of mammalian pregnancy ensue. A "luteal-like" phase only occurs if conception takes place but is later terminated by embryonic or fetal demise or if mating occurs but fertilization is not successful. Thus, as a common event, the luteal phase is confined to human beings. As such, the luteal phase should be viewed as an unusual time during which the uterus and perhaps all of maternal physiology is actively engaged in sustaining the development of the fertilized egg if, in fact, conception has occurred.

Summary

The physiological responses of women to altitude have received comparatively little study until recently. Of interest is whether women and men differ in their physiological response to high altitude and whether differences are due to circulating ovarian or gonadal hormones or to sex differences unrelated to levels of circulating hormones. For such studies, it is important to recall that the determination of sex involves considerable evolutionary, genetic and developmental complexity. Studies of acute and chronic response to high altitude provide useful case studies for examining the general mechanisms and evolutionary significance of the influences of sex and sex hormones.

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CHAPTER 2

ENERGY REQUIREMENTS OF WOMEN AT ALTITUDE

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Introduction:

Energy balance is mandatory for maintenance of body weight and composition.¹² Failure to maintain energy intake commensurate with energy expenditure will result in weight loss and mobilization of energy stores as an alternative fuel source for cellular activities. Failure to maintain energy balance, and consequent weight loss, is a common thread throughout the literature describing human research at elevations above 1000 m.^{5, 13, 14, 17, 21, 24, 26, 30, 34, 35, 45, 46, 51, 54, 62, 63, 65}

The purported causes of energy imbalance at altitude are well documented, including anorexia (failure to ingest sufficient energy,^{5,13,14,21,24,26,29,30,34,35,45,46,51,54,62,63,65}), increased needs (either as increased basal needs^{7,8,21,23,26,43,45,54} or increases in physical activity due to military service or climbing activities^{5,21,30,45,46,47,53,62,63,65}), or malabsorption of the nutrients consumed.^{5,17,21,45,63}

However, these previous references have been limited primarily to one gender, men. Although, gender differences have been documented at altitude for many years,¹⁹ we know very little about the components of energy balance in women at altitude. Women live and reproduce at altitudes above 2500 m; they have accompanied men on sojourns to that and higher altitudes. However, their experience related to energy balance has been either undocumented, encased in the data presented on men,^{23,46,54,63} or presented as very small samples (n=1²²; n=2⁵⁵). Thus, we are left with the question, what does a woman require to maintain energy balance and body mass at high elevations? Is energy imbalance as prevalent in women traveling to high altitudes as it is in men? Are the purported causes of energy imbalance in women similar to those in men? What evidence is available?

Elements of Energy Balance at Altitude:

Energy balance is the algebraic sum of net energy intake minus energy output in the form of basal energy needs (the energy required to maintain body functions in a sedentary state), energy required for metabolism of meals (thermic effect of food), energy required for specific activities (e.g. job related energy expenditure), and energy required for random activity (fidgeting⁴⁸). Based on what we know from studies on men, some, but not all, of these parameters are altered at altitude.

Net energy intake is generally found to be depressed at altitude, primarily because of diminished intake, with little or no contribution from malabsorption. A

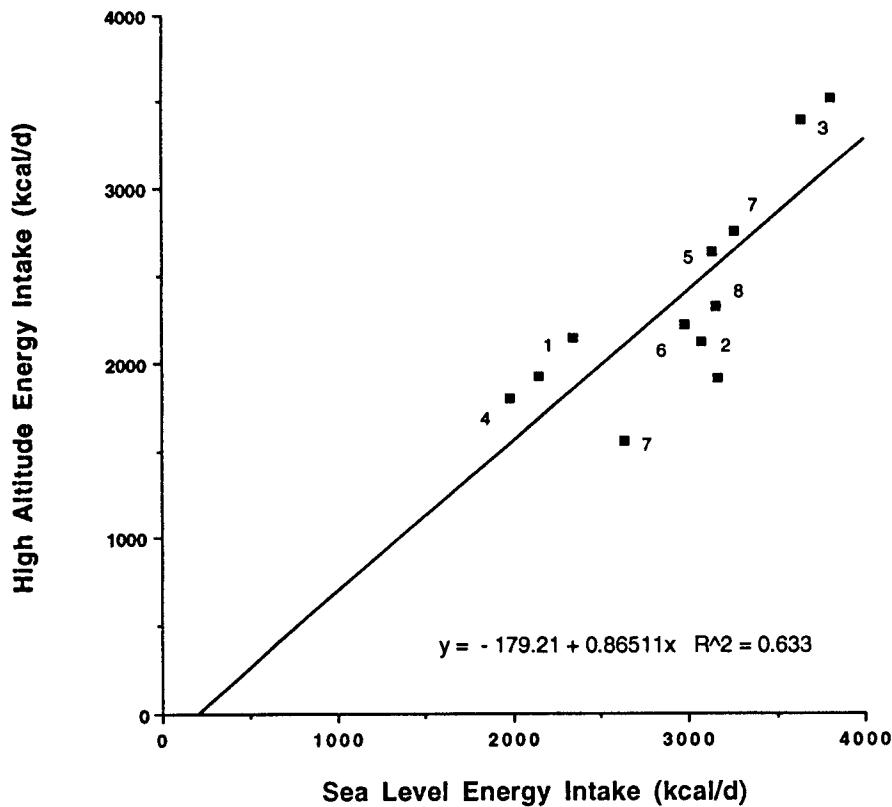


Figure 1 Relationship between sea-level energy intake (kcal/d) and high-altitude energy intake (kcal/d) compiled from studies involving *ad libitum* food intake and providing accurate measures of both parameters. Numbers next to boxes indicate references used: 1 (ref 14); 2 (ref 35); 3 (ref 13); 4 (ref 27); 5 (ref 53); 6 (ref 5); 7 (ref 24); 8 (ref 51). Reproduced with permission from ref 10.

review of studies reporting reliable sea level and altitude intake data suggests that energy intake at altitudes greater than 2500 m is chronically depressed below sea level intake by about 180 kcal (Fig. 1,¹⁰). Early work on the effect of altitude on gastrointestinal physiology suggested that gross energy intake may be further hampered by malabsorption of carbohydrate,⁵ protein or fat.^{5,45} However, any possible negative effect of altitude on gastrointestinal function which may result in physiologically significant malabsorption of nutrients below 5500 m has been more than adequately refuted by a number of studies for fat,^{8,31,47} and protein.^{8,33} Malabsorption of carbohydrate, although not found at 4300 m,⁸ is still somewhat in question at altitudes greater than 5500 m,¹⁷ although the effect, if present, is probably of minimal significance to overall energy balance.

Basal energy needs may be transiently increased by as much as 20 to 30% during the first days at altitude (Fig. 2,⁴³), and the rise in basal need appears to be somewhat altitude dependent (see ref 9 for review). Although the methods used for determination of basal energy need may vary, with some studies measuring true basal metabolic rate (before rising in the morning, BMR), and others measuring resting metabolic rate (sitting quietly for 30 minutes before measure, RMR), all show the

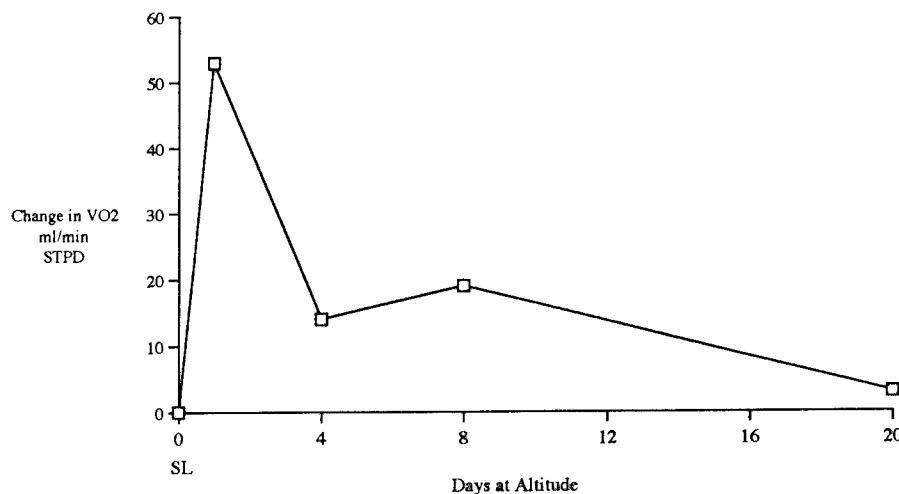


Figure 2 Resting metabolic rate in men exposed to 4300 m for three weeks with *ad libitum* dietary intake. Redrawn by permission from ref 43.

same trend. In most studies, however, these elevated basal needs fall to near sea level values within 21 days,⁴³ possibly consequent to decreases in lean body mass, the metabolically active tissue in the body. Energy expended in response to meals is slightly elevated⁵⁴ upon acute exposure to altitude, but appears to drop with acclimatization. The energy required to perform work (measured as oxygen consumption) at a given power output has been shown to be constant across several weeks at altitude when measured under circumstances of constant load.^{6,61}

Overall energy requirements for men at altitude, then, depend primarily on the increase in energy requirement resulting from increases in basal needs (usually increased about 300 to 400 kcal/d above sea level), and the needs accompanying the activities performed. Recent studies^{30,46,62,63} using the technique of double labeled water (DLW) to estimate the energy requirements of men at altitude have the advantage of being able to estimate total energy expenditure (which mimics need) in the free-living, normally active state. However, some of these same studies^{30,46,63} have shown that the intake-balance method of estimating energy requirement is just as reliable for the group as the expensive DLW method, as long as assumptions related to the composition of body stores (water content and energy equivalent of fat and lean) used to balance energy needs are thoughtfully made.⁴⁶ These and other studies have documented energy needs in climbers ranging from 3200 kcal/d (1.6 to 2.4 times sea level BMR) in 4 men (and 2 women, undifferentiated in report) resting at 6642 m⁶³ and 3250 kcal/d (2.2 times sea level RMR) in men climbing near the summit of Mount Everest⁶² to 4600 kcal/d in men climbing to 8000 m.⁴⁶ Using DLW in military troops, energy need ranges from 3800 kcal/d (2.1 times basal needs) in individuals performing routine activities at about 3500 m,⁶⁵ to 4600 kcal/d (3.5 times sea level RMR) in soldiers performing strenuous winter exercises at 2500 to 3100 m.³⁰ In all of these instances, altitude energy intake was reported to be less than that at sea level (and thus less than altitude need), and weight loss was significant, ranging from 100 to 330 gm/d (see ref 10 for review).

Thus, the failure to attain energy balance in men can be attributed to the lack of adequate intake and an increase in energy need. Work by Butterfield and co-workers on Pikes Peak has shown that diligence in enforcing energy intake sufficient to cover the increase in basal energy needs will result in a cessation of weight loss in sedentary men living at 4300 m for three weeks.^{7,8} Total energy requirement for weight and body composition maintenance at altitude was found by these investigators to be 2.05⁸ or 2.27 times sea level BMR in these relatively sedentary men. In these studies, Butterfield and colleagues found that with adequate energy intake, basal energy needs remained elevated throughout the three week sojourn (Fig. 3,⁷) unlike other studies where basal needs fell.⁴³ They attributed this elevation to the maintenance of lean body mass (nitrogen balance was maintained under circumstances of adequate energy intake,^{7,8}) and an increased thermic effect of food due to the elevated energy intake required by their protocol (subjects consumed 300-400 kcal/d more at altitude than at sea level to maintain body weight,⁸). The final basal energy expenditure values (about 260 ± 20 ml O₂/min) were similar to those reported by others^{21,44} in native dwellers at elevations above 2500 m.

Under the circumstances of energy balance, Butterfield and colleagues were able to show that the primary metabolic fuel used at altitude during both rest and exercise was carbohydrate^{6,50} rather than fat,⁴⁹ as has been inferred from other studies in inadequately fed men.⁶⁶

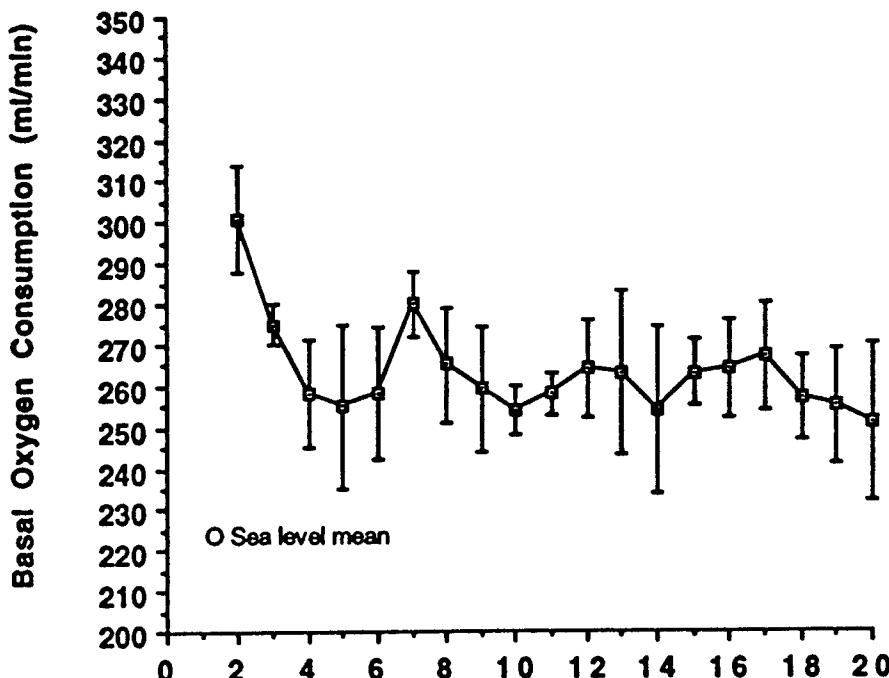


Figure 3 Basal metabolic rate in men exposed to 4300 m for three weeks with dietary intake enforced to cover increased basal needs. Reproduced with permission from ref 7.

Impact of the Menstrual Cycle on Energy Requirements in Women:

The work on energy requirements of men at altitude gives us a starting point for addressing the possible changes in need for women living temporarily at altitude. However, the study of energy requirements in women is complicated by the menstrual cycle. Fluctuations of the ovarian steroid hormones estradiol (E2) and progesterone (P4) concurrent with the menstrual cycle have been shown to affect both BMR and energy intake at sea level, and consequently may affect response in those parameters at altitude.

Changes in BMR over the menstrual cycle have been examined since the early 1920's,^{4,42,52,58,64} and most investigators have found BMR to fluctuate in concert with E2 and P4 secretions. BMR is generally found to be lowest in the follicular phase (moderate E2, low P4), and highest in the latter half of the luteal phase (high E2, high P4) (Fig. 4). Bisdee, et al⁴ reported that BMR increased by 56 kcal per day (6.1%) at its zenith in eight women studied in a metabolic unit. Solomon, et al⁵² documented an even greater increase of 360 kcal/d (approximately 25%) in BMR during the luteal versus the follicular phase in 6 women confined to a metabolic ward across several menstrual cycles. Sleeping metabolic rate (SMR) was increased 7.7% during the post-ovulatory phase of the menstrual cycle in 16 women studied in a respiratory chamber.⁴² Bisdee and co-workers⁴ in reviewing the literature, identified significant variation in the response, and suggested that this variation could be attributed to failure to control dietary intake resulting in fluctuations in BMR in response to energy content or diet composition. Failure to verify menstrual cycle phase in

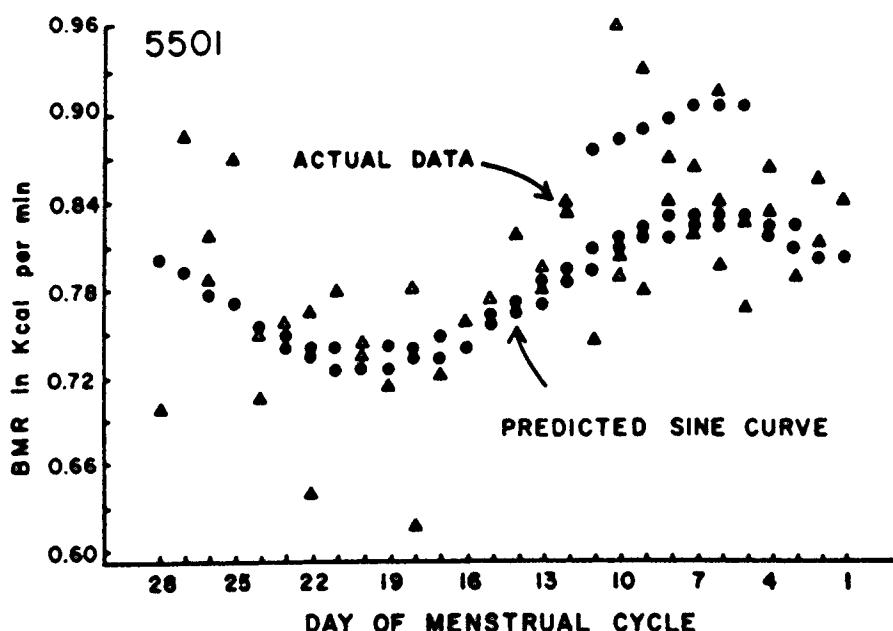


Figure 4 Basal metabolic rate measured in a representative woman confined to a metabolic ward over three complete menstrual cycles. Dietary intake was controlled and adequate to maintain body weight. Sine waves (closed dots) were fit to the data to demonstrate periodicity. Reproduced with permission from ref 52.

some studies may have led to further variability in the data. The peak in basal energy expenditure during the luteal phase is commonly attributed to the thermogenic effect of progesterone,³ although Calloway and colleagues were unable to verify a relationship between hormone levels and metabolic factors (BMR or protein need) in their study on 6 women studied with constant food intake under metabolic unit conditions.¹¹ Webb⁵⁹ found that the increase in basal needs during the luteal phase of the menstrual cycle translated into an increase in total energy expenditure of 8-16% in women measured in his laboratory.

It should be noted that fluctuation in basal energy expenditure across the menstrual cycle is not a universal finding. Westrate,⁶⁴ studying 23 women over a 3 month period, was unable to show this variation in energy expenditure across the menstrual cycle when measuring RMR. However, he notes that his subjects watched films while the measurement was undertaken, which might have elevated energy expenditure through excitement, and that failure to control diet may lead to additional variability in BMR for both men and women.

Of further concern in the study of BMR and how it may affect total energy requirements in women at sea level or altitude are recent reports suggesting that this parameter is lower in women than in men for reasons other than differences in fat mass and fat free mass. Arciero, et al¹ showed a 3% lower RMR in 194 women 18 to 81 years as compared to 328 men 17 to 80 years even after correction for fat-free mass, fat mass, activity level and gender. Ferraro, et al¹⁸ showed similarly that total sedentary energy requirement was 124 kcal/d higher in men than women, even when adjusting for fat free mass, fat mass and age. Neither of these investigators has been able to offer an explanation for this difference. Weinsier, et al⁶⁰ suggested that variation in BMR may be due to differences in the proportion of fat free mass contributed by muscle and non-muscle organs, the latter contributing more to the fat free mass of women.

Fluctuations in energy intake across the menstrual cycle have been found by some to mimic this periodicity in energy need. Many investigators (see ref 56 for review) have shown *ad libitum* energy intakes to be lowest during menstruation and ovulation, and highest during the late luteal phase. Martini, et al³⁸ found an increase of 159 kcal/d in energy intake during the midluteal phase of the menstrual cycle. Of particular note in this experiment was their careful attention to the time of ovulation as determined by urinary luteinizing hormone excretion. The increase appeared to be accomplished by uniformly higher intakes of carbohydrate, protein and fat. Barr, et al² found a similar increase in a group of women for whom they documented ovulation using ovulation kits. Most reports have found this elevated energy consumption during the luteal phase to range from 100 to 500 kcal (4 to 40% of basal needs) over intake during the follicular phase. Thus, this apparent increase in appetite corrects for much of the increase in basal energy needs found during the same phase. It should be noted, however, that this finding of increased energy intake during the luteal phase is not universal. Other investigators^{20,57} have been unable to verify this effect.

Energy Requirements of Women at Altitude:

The data on energy requirements in women at altitude fall into two categories, those from studies of chronic altitude dwellers, and those from women acutely exposed to altitude.

In the 1930's and 1940's, several investigators addressed the effect of chronic altitude exposure on the basal energy needs in women residing permanently at altitude.^{15,16,19,32,36,37,39,40,41} McKittrick, et al⁴¹ measured the RMR in female residents of Laramie, Wyoming (2178 m), and found the values to be higher than published data for women residing below 300 m. McCrery and colleagues^{39,40} found that the RMR of 100 women residents of Lubbock, Texas (975 m) fell between the values published for residents of Laramie and those of women at 300 m. However, Lewis published data³⁶ on 43 women residents of Denver, Colorado (1609 m) disputing these results. He found no difference between the data for the Denver residents and those published for sea level residents. His explanation for the discrepancy between his work and others was the small number of subjects studied at altitude. He supported his contention that there was no effect of altitude on resting energy requirements by taking a group of women and measuring their RMR in Stillwater, Oklahoma (approximately 300 m), Denver (1608 m) and Eldora, Colorado (2650 m) after about 3 weeks of acclimatization to each altitude.³⁷ He found no difference in RMR in the women, regardless of the elevation at which it was measured.

More recently Cudkowicz, et al¹⁵ compared resting oxygen consumption in women native to 3600 m in La Paz, Bolivia to that of "newcomers" to the region (defined as women residing in the area for several weeks) and found no significant difference between the groups when the data were expressed per kg body weight (the natives were significantly smaller than the "newcomers"). Further, when the "newcomers" were taken to a higher elevation (5200 m) there was no measurable increase in basal oxygen consumption. Conversely, Dahr, et al¹⁶ showed a significantly higher basal energy expenditure in 21 Kashmiri women living at altitude as compared to sea level Indian natives. Kashiwazaki, et al³² evaluated total energy requirement of twenty three 18-65 year old Aymara natives living at 4000 to 4200 m in Bolivia and found no differences between the needs of these individuals (2350 kcal/d) and those living at lower altitudes. The general consensus of these studies is that women born and living at high altitude weigh less than their sea level counterparts, and thus require less oxygen. This diminutive body size, however, may be influenced by many factors besides hypobaric hypoxia, such as malnutrition, socioeconomic status, and access to resources, medical and otherwise.

Thus, the effect of altitude on basal and total energy requirements in women is unclear. To date, the most thorough studies of energy balance in women acutely exposed to high altitude have been performed by Hannon and colleagues. In two separate studies, these investigators evaluated basal energy needs, food intake, and maintenance of body weight and body composition in two groups of women spending the summer on Pikes Peak (4300 m) in Colorado. The first study^{28,29} involved eight college-age women who were taken from the University of Missouri to the top of Pikes Peak for a 3 month stay. The degree of anorexia experienced by these women during the first week at altitude was found to be significantly less than that seen in men. Weight loss over the first week was reported to be less than 1 kg, and was much less than weight lost by men in the same facility. Energy intake had returned to sea level values by 7 days (Fig. 5), but careful perusal of the data²⁵ shows weight loss continued past the first week, although at a very slow rate.

Food intake, basal and total energy needs, body weight, and nutrient balances were studied more closely in a subsequent study on an additional eight college-aged women taken from the University of Oregon to the top of Pikes Peak^{26,27} within 8

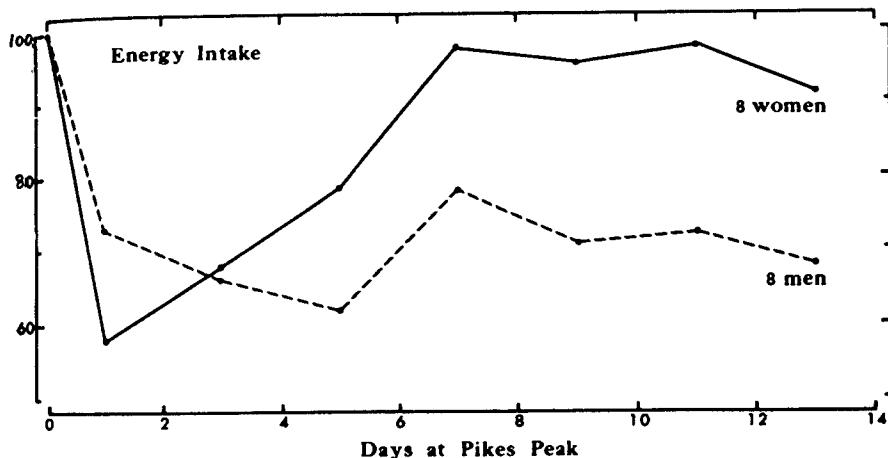


Figure 5 Effects of high altitude (4300 m) exposure on energy intake in men and women. Levels are expressed as percent of low altitude control intakes (2,980 kcal/d for men and 1,980 kcal for women). Reproduced with permission from ref 29.

hours of leaving Oregon. Despite attempts to prevent cachexia, the women lost an average of 1 kg over the first 7 days of exposure and spent the first three days in negative nitrogen balance. These authors noted a 28% rise in BMR (measured in the morning before rising) during the first three days of exposure. By the sixth day at altitude, however, BMR was not significantly different from sea level values (Fig. 6). Despite attempts to accommodate the women by providing palatable foodstuffs, *ad libitum* energy intake was significantly depressed below sea level values (approximately 700 kcal/d deficit) over the first 3 days. However, appetite appeared to increase gradually from that point, and by day 7 at altitude the women had returned to an intake not significantly different from sea level (Fig. 5). However, the mean

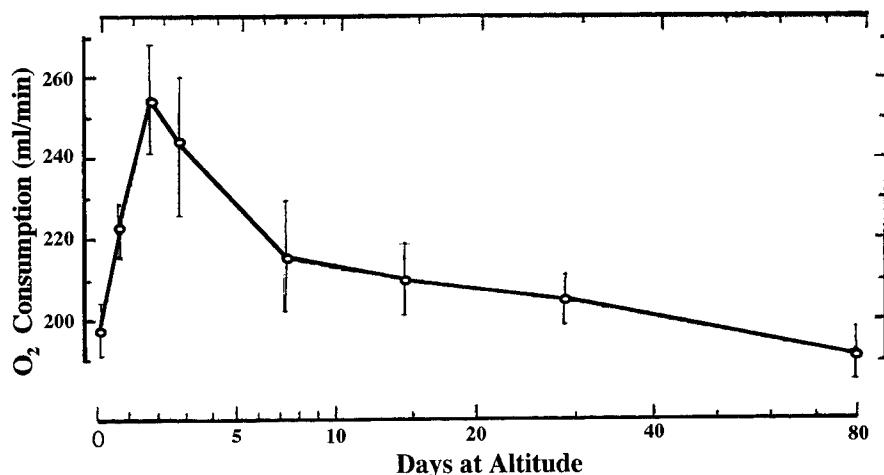


Figure 6 Basal metabolic rate in women exposed to 4300 m for three weeks. Reproduced with permission from ref 26.

intake was still 170 kcal less than the sea level average, the value found to be the "rule" in many studies (Fig. 1¹⁰). In addition, Hannon and Sudman found that nitrogen excretion increased beyond intake during the first three days, then reversed and came close to approximating intake after the fourth day of exposure. However, if nitrogen (N) balance were calculated according to standard methods (N balance = N intake - [urinary N + fecal N + miscellaneous losses of N]), the subjects would be seen to be in continued negative nitrogen balance, and the loss of 1 kg of weight over the time of study becomes predictable. The return of BMR, nitrogen balance, and energy intake toward sea level values occurred more rapidly in these women than has been seen commonly in men.^{14,43} Based on these data, as well as data demonstrating that women recovered more quickly from acute mountain sickness,²⁹ Hannon²⁵ suggested that women may actually acclimatize better than men. The caveat should be noted, however, that total energy requirement for the women was about 1000 kcal/d less than for the men, making attainment of adequate energy intake more feasible.

Hannon's studies, as well as those involving the chronic altitude dwellers, were performed on women allowed to eat at will, and were not controlled for menstrual cycle. This situation may explain some of the discrepancies between the results of different studies, and may be, at least in part, the source of the frustration in interpreting these varied results. Based on the observations of Butterfield, et al,⁸ that maintenance of an energy intake adequate to maintain body weight and nitrogen balance in men acutely exposed to altitude is associated with a sustained elevation in BMR (and thus elevated energy need), the possibility exists that a similar maintenance of elevated BMR will be found in women, as is seen in some of the studies on chronic altitude dwellers. Such a maintenance of elevated energy need would increase the total energy requirement for women living at altitude. That such an effect will be confounded by menstrual cycle phase seems inevitable, given the data demonstrating a cycle phase effect upon at least two of the elements of maintenance of energy balance, basal energy needs and energy intake. Thus, it becomes important to determine the energy requirements for maintenance of body mass in women at altitude within the context of the menstrual cycle.

We have recently completed a highly controlled study in which sixteen women were studied both at sea level and at Pikes Peak (4300 m) during either the follicular (n=7) or luteal (n=9) phase of the menstrual cycle. By individually adjusting energy intake to accommodate changes in expenditure, we were able to prevent weight loss. Basal metabolic rate followed a pattern similar to that seen by Hannon and Sudman, but energy requirement for weight maintenance remained elevated and continued to rise throughout the 12 days of the altitude sojourn.

Conclusion:

In women, net energy balance at altitude may be attributed to the same elements as those found in men: energy intake, basal energy expenditure, thermic response to food, and energy required for activity. The available data suggest that although both intake and basal expenditure may be transiently altered with acute exposure to altitude, women regain equilibrium more rapidly and more easily than men. However, as with the literature on men, the data in women are inconsistent among reports. Failure to control for factors which alter energy needs in women, even at sea level, may account in part for this difference. The energy balance equation in women is

complicated by fluctuations in the metabolic rate and energy intake concurrent with the menstrual cycle. In addition, gender differences may exist with respect to RMR and total energy requirement not explained by body size or composition. Thus, the interplay of factors comprising energy balance in women at high altitude requires more investigation. Studies enforcing intake (and thus maintaining body composition) and accounting for menstrual cycle changes will help elucidate the most appropriate method to ensure adequate energy intake for women at altitude.

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CHAPTER 3

WOMEN AT ALTITUDE: SUBSTRATE UTILIZATION DURING REST AND EXERCISE.

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1. Introduction

The purpose of this chapter is to summarize what is known regarding substrate utilization in women, at rest and during exercise, during acute and chronic exposure to high altitudes. The focus will be on the uptake and oxidation of substrates to produce energy at the whole-body level. Discussion of nutrient intake, storage, and metabolism at the cellular level have been omitted for reasons of space and clarity. Hopefully, these topics will be reviewed in forthcoming volumes. By necessity, much of this review comprises data collected in male subjects, since so few data are available from studies including women. Potential influences of gender, especially with respect to the ovarian hormones estrogen and progesterone, on rest and exercise substrate use at sea level are outlined. Finally, we present a rationale for why women may respond metabolically to high altitude differently than men.

2. Basic principles of substrate utilization

Substrate use during rest and exercise

At rest, in the post-absorptive (fasting) state, humans obtain 60-90% of their energy requirements by oxidation of fatty acids, with the remainder representing mainly the obligate use of blood glucose by the nervous system and erythrocytes²⁸. Blood glucose is almost entirely derived from breakdown of liver glycogen and gluconeogenesis, with very little muscle glycogen utilized at rest²⁸. After a meal, the balance of substrate oxidation shifts to increasing carbohydrate use in response to a rise in plasma glucose and insulin concentrations, with 50-95% of energy requirements attributable to glucose oxidation²⁸. Two to 4 hours post-meal (depending on meal size and composition), the body gradually shifts back to predominantly fat oxidation to meet its energy needs²⁸.

Substrate utilization during exercise is dependent on both nutritional (proximity to the last meal, composition of the recent diet, etc.) and physiological factors (Table 1). Of these, exercise intensity is the overwhelming determinant of substrate use as there is a strong direct relationship between increasing exercise intensity and the percent of energy derived from carbohydrate^{12,69}. Composition of the recent diet, fasting, endurance training, or increasing exercise duration (as muscle glycogen con-

tent diminishes) can all shift substrate use at a given absolute exercise intensity^{12,32}. Environmental factors such as heat and hypobaric hypoxia can also alter the fuel mix that the body oxidizes for energy.

Table 1

FACTORS AFFECTING SUBSTRATE UTILIZATION*	
Endurance Training and Cardiovascular Fitness	
Exercise Intensity and Duration	
Muscle Morphology and Histology	
Fiber type distribution and enzyme activity	
oxidative enzymes	
glycolytic enzymes	
Hormones	
Stress	
catecholamines	
cortisol	
growth hormone	
thyroid hormone	
Gonadotrophic	
testosterone	
estrogen	
progesterone	
Metabolic	
insulin	
glucagon	
Cellular mechanisms	
Insulin receptors	
Glucose transporters	
Diet and Nutritional Status	
Body Composition and Fat Distribution	
Environmental Factors	
Altitude (hypoxia)	
Heat	
Cold	
Blood Flow Distribution / Tissue Perfusion	

*Adapted from Ruby (70). Also see (58) for more detail.

3. Importance of energy balance

The confounding effects of energy balance on substrate utilization would be difficult to overstate. For an extensive review of energy balance at high altitude, the reader is referred to the chapter by GE Butterfield and JT Mawson appearing in this volume. A brief summary of how the negative energy balance often encountered in people traveling to high altitude affects substrate utilization is included below.

Impact of negative energy balance on substrate use

Negative energy balance, which results in body weight loss over time, has a dramatic impact on substrate oxidation. Concentrations of fatty acids in the blood rise

and insulin levels fall, resulting in greater oxidation of fatty acids and decreased uptake of glucose by muscle and fat cells^{5,65,66}. Use of amino acids as a metabolic fuel is also increased when energy balance is negative, as body protein (muscle) is degraded and liberated amino acids are either oxidized for energy or converted to glucose by the liver to help stabilize blood glucose levels^{8,15}. The net result of these adaptations to insufficient energy intake is decreased reliance on carbohydrate as a metabolic fuel as glucose is conserved for use by the tissues of the nervous system. Metabolic studies at altitude complicated by negative energy balance would result in the appearance of a greater reliance on fat which may not reflect a "true" response to hypobaric hypoxia.

4. Summary of Literature on Men at Altitude

Studies without rigid dietary control

The literature is ambivalent regarding the acute and chronic effects of high altitude on substrate utilization in men. As noted in a review by Houston, fasting glucose concentrations tend to be lower during chronic exposure to high altitude.⁴⁰ There is a hypoglycemic effect of negative energy balance however, and most of the work cited by Houston is in high-altitude sojourners who were not on a controlled diet and thus were likely to consume considerably less energy than they expended^{9,16}. Young et al. reported concentrations of serum glycerol that were, relative to sea level, 2-fold (at rest) and 3-fold (immediately after exercise at 85% VO₂max) higher after 18 days at 4300 meters.⁷⁶ Free fatty acid concentrations were unchanged from sea level, which the authors interpreted to indicate that fatty acid uptake by peripheral tissues was increased at high altitude. Respiratory exchange ratios were lower at altitude, in accord with the substrate data. Resting muscle glycogen content was lower after chronic but not acute altitude exposure. During exercise, net muscle glycogen utilization was 42% lower after chronic altitude exposure, supporting the inference that increased fat oxidation spared muscle glycogen.⁷⁵ These data confirmed results from other studies showing increased lipolysis in men exposed to hypoxic conditions.⁴³

Conversely, Johnson et al. used an isotopic tracer technique ([¹⁴C-glucose]) and reported increased glucose turnover, compared with sea level, in 7 men studied after acute exposure (40 hours) to 4300 meters.⁴² Isotopic analysis of ¹⁴CO₂ showed that glucose oxidation was also elevated at altitude. In accordance with the kinetic data showing increased carbohydrate use, respiratory exchange ratios were higher after acute altitude exposure. Energy intake was not controlled in this short-term study and although weight loss probably was unmeasurable, subjects were likely in negative energy balance.

In response to the confusion generated by the disparate results reported in the literature, studies on Pikes Peak were conducted in 1988 and 1991 which included aggressive feeding to eliminate or minimize the confounding effects of weight loss on substrate utilization at high altitude.

Studies with rigid dietary control

Fasting metabolism

Fasting blood glucose concentrations are generally 5-10% lower after exposures to high altitude lasting longer than a week^{11,68}, indicating the results reviewed by

Houston⁴⁰ are true adaptations to hypobaric hypoxia and not to starvation. Decreased circulating glucose levels are probably not explainable by depleted liver glycogen in these subjects who were fed a weight-maintaining diet composed of >55% carbohydrate. Circulating levels of the glucoregulatory hormones insulin and glucagon did not change in a physiologically meaningful way after altitude acclimatization^{26,50,68}, but acute effects were noted, including an increase in fasting insulin concentration after 2 days at 4559 meters^{26,50}. Plasma concentrations of cortisol were not different from sea level values after 7 days at high altitude although again, acute changes have been observed, for example an increase in fasting cortisol concentrations after 2 days at 4559 meters^{26,50}. Blood levels of epinephrine (acute rise then fall back toward sea level) and norepinephrine (constant rise throughout altitude exposure) were greater at high altitude relative to sea level^{11,56,68,72} but this change in the hormonal milieu would be expected to increase rather than decrease blood glucose concentrations. Subjects at altitude may be more insulin-sensitive in peripheral tissues (more blood glucose taken up by tissues at a given plasma insulin concentration) or in the liver (less glucose released to the blood at a given insulin concentration). The practical significance of altered insulin sensitivity in the fasted state is likely to be minimal however, since most glucose disposal in this condition is non-insulin-dependent and mediated by mass action. In summary, the mechanism responsible for mild hypoglycemia observed at high altitude is unclear but a result may be to reduce resting glucose utilization, conserving glucose for other activities.

In two separate studies (taking place in 1988 and 1991) on Pikes Peak, Brooks and coworkers studied glucose utilization at sea level and after 1 day and 21 days at 4300 meters.^{11,68} In 1988, glucose turnover rates measured using isotope dilution (to assess whole body glucose turnover) were the same as sea level after 1 day at 4300 meters, but higher after 21 days.¹¹ In the 1991 study however; whole body glucose turnover was significantly elevated after 1 day at high altitude (1.98 mg/kg/min) relative to sea level (1.47 mg/kg/min), with intermediate values after 21 days (1.67 mg/kg/min).⁶⁸ Leg glucose uptake (LGU), measured using the limb balance technique, did not follow the same pattern however. LGU was elevated after 1 day at 4300 meters but had returned to baseline by 21 days.

In a study taking place on Monte Rosa, basal glucose turnover (whole-body) was the same after 2 or 7 days at 4559 meters compared with sea level.^{26,50} Taken together, these data suggest that basal glucose utilization is the same or higher than sea level values after acute (2-7 days) altitude exposure, and consistently higher than sea level after acclimatization. Which tissues are actually responsible for the increased glucose utilization at rest is unclear.

With subjects in energy balance, blood concentrations of free fatty acids rise approximately 2-fold upon acute exposure to altitude and remain elevated during acclimatization.⁶⁷ Glycerol levels in the blood follow a similar pattern. Using the limb balance technique to assess fatty acid uptake by the legs, Roberts et al. found that resting fatty acid utilization was the same after 2 days (.07 mmol/min) and lower after 21 days (.01 mmol/min) at 4300 meters compared with sea level (.08 mmol/min).⁶⁷ These data imply that increased concentrations of free fatty acids and glycerol in the blood do not necessarily result in greater use of fat as an oxidizable substrate, and in fact, fatty acid consumption is lower after acclimatization to high altitude.

Based on carefully controlled studies using both isotopic blood tracers and arteriovenous differences across a muscle bed, it appears that in men glucose utilization is increased and blood fat utilization is decreased after acclimatization to high altitude. Acute effects are more variable and their magnitude and direction may depend on unpredictable changes in plasma concentrations of cortisol, insulin, catecholamines, and other glucoregulatory hormones.

Post-prandial metabolism

At high altitude, compared with sea level, a lower blood glucose response to an oral or intravenous glucose load is consistently found.^{29,61,62} This observation could be explainable by incomplete or delayed absorption of carbohydrate from the intestinal lumen, although Chesner et al. found no evidence for carbohydrate malabsorption at high altitudes.²² Reduced levels of liver glycogen could lower glucose output by the liver and explain the lower glucose response, especially in poorly nourished subjects. Increased glucose disposal after a meal might result from a greater insulin response, or enhanced sensitivity of peripheral tissues (muscle and fat) to the insulin signal. Alternatively, increased hepatic insulin sensitivity could lower glucose export from the liver. When Larsen et al. measured insulin sensitivity directly using the euglycemic, hyperinsulinemic clamp technique, they found it was lower, not higher, after 2 days (4.3 mg/kg/min) and 7 days (7.2 mg/kg/min) at 4559 meters relative to sea level (9.6 mg/kg/min).^{26,50} Their method primarily evaluates peripheral insulin sensitivity and the effects of altitude on the hepatic response to glucose and insulin are unknown. Almost nothing has been reported regarding the post-prandial metabolism of fat and protein at high altitudes.

The absorption, trafficking, and storage of nutrients at high altitude has been relatively understudied and could have potent effects on substrate utilization. Although the methodology required to carefully address the pertinent questions is complicated by the presence of non-steady-state conditions, this is a fertile area for future research.

Exercise

As previously mentioned, before the series of studies on Pikes Peak which incorporated aggressive dietary control,^{10,11,13,17,56,67,68} there was general consensus that exposure to hypobaric hypoxia resulted in a greater reliance on fat as an exercise substrate.^{72,76,77} In male subjects who are kept at their sea level body weight however, a very different response has been observed.

Brooks et al. used isotopic dilution of infused [6,6²H] glucose to study blood glucose utilization in men cycling at 50% of their sea level VO₂peak (corresponding to 65% of altitude VO₂peak). After 1 day at 4300 meters, exercising blood glucose uptake during exercise was 26% higher than sea level values, and was significantly elevated after 21 days as well.¹¹ In a similar study which incorporated direct measurement of substrate balance across the working leg in addition to whole-body glucose kinetics, the following pattern was observed:

Measure	Sea Level	2 days at 4300m	21 days at 4300m
Glucose Turnover Rate (mg/kg/min)	3.04	4.16	3.57
Leg glucose uptake (mmol/min)	0.57	1.40	0.96

There was excellent agreement between the 2 independent measures of blood glucose utilization, with a large rise in glucose use upon acute exposure which returned partway to sea level values after 21 days of acclimatization. During exercise, the increase in leg glucose uptake accounted for all of the rise in whole-body glucose turnover.¹³ Because the time course of the changes in glucose uptake paralleled fluctuations in plasma epinephrine concentrations, it was anticipated that blocking the beta-adrenergic system might attenuate the altitude effects. However, dense beta-blockade with propanolol potentiated the altitude effects, with even greater rises in glucose uptake than with placebo.

Concurrent with the study of glucose kinetics, this group also used the tracer technique and limb balance to assess free fatty acid uptake in the same conditions.⁶⁷ Results are summarized in the table below:

Measure	Sea Level	2 days at 4300m	21 days at 4300m
Free fatty acid uptake (mmol/min)	0.72	0.48	0.28
Free fatty acid consumption (mmol/min)	0.90	1.07	0.25

In contrast with the glucose data, free fatty acid uptake declined over the course of altitude exposure with the lowest values on day 21. Free fatty acid consumption was not different from sea level on day 2 but decreased markedly by day 21. Propanolol potentiated the effects of altitude on fatty acid metabolism so that both uptake and consumption were lower at all timepoints in the beta-blocked condition.

Taken together, the results of these studies clearly show a shift in exercise fuel use toward blood glucose and away from blood-borne free fatty acids after acclimatization to 4300 meters altitude. These data are not necessarily incompatible with the marked decrease in muscle glycogen utilization reported by Young et al. during 30 minutes of exercise at 80-85% VO₂max after 18d at 4300m.⁷⁶ As mentioned above, Young et al. interpreted their data to indicate a greater reliance on fat during exercise but their observations are also consistent with a preferential use of blood glucose rather than muscle glycogen after acclimatization to high altitude.

Summary of Literature on Men

Several other lines of evidence support the hypothesis that blood glucose is utilized to a greater extent at high altitude compared to sea level. Holden et al. found that regional glucose uptake rates by the heart were higher in Quecha and Sherpa subjects, who are adapted over generations to high altitude, than in sea level residents.³⁹ Cartee et al. found that exposure to hypoxia increased glucose transport in perfused rat hindquarter preparations.²⁰ Strips of muscle from insulin-resistant sub-

jects take up more glucose from the culture medium when incubated under hypoxic conditions relative to normoxia.³ In other physiological states characterized by reduced ability to use oxygen, such as iron deficiency and anemia, glucose turnover and oxidation are increased during exercise (both) and at rest (iron deficiency only).⁷ The increased dependence on glucose during hypoxia does not appear to be strongly regulated by gross changes in the concentrations of glucoregulatory hormones, cortisol, or the β -adrenergic limb of the sympathetic nervous system. Hypoxia-induced adaptations at the cell receptor or post-receptor level are likely to be involved in the preferential use of glucose as a fuel in men acclimatizing to high altitude.

Hochachka³⁸ and Brooks⁷ have theorized that increased dependence on glucose as a metabolic fuel in hypoxic conditions is an adaptive strategy to maximize energy production per mole of oxygen. Oxidation of carbohydrate alone produces 5.05 kcal per liter of oxygen as compared with 4.68 kcal/liter O_2 when fatty acids are burned exclusively. As a matter of practical importance however, it is difficult to understand how the relatively minor shifts in fuel selection observed at high altitude could have a major impact on the efficiency of oxygen utilization. A rise in the percent of energy derived from carbohydrate that far exceeds the magnitude of change observed at high altitude, for example from 60% to 80% of total energy expenditure, only changes fuel economy from 4.899 to 4.973 kcal/L O_2 , an increase of 1.5%. In contrast, the same change in fuel selection increases carbon dioxide production per liter of oxygen consumed by 7%. In 1974, Johnson et al. speculated that shifting substrate utilization toward carbohydrate could result in higher arterial pCO_2 concentration and induce a better ventilatory adaptation to high altitude.⁴² Although this is a tantalizing idea, it remains untested.

5. Previous data on substrate utilization in women at altitude

The only data available regarding how women respond to high altitude in terms of substrate use were provided by Hannon.³⁶ Eight women showed a decrease in fasting plasma glucose concentrations and an increase in plasma free fatty acid concentrations during 14 days exposure to 4300 meters. These changes were transient, free fatty acid levels returned to sea level values after 7 days and glucose concentrations approached baseline after 14 days. Because the rise in plasma free fatty acids was more robust and prolonged in men (still increasing even after 14 days), the author felt it was likely that men were using body fat to a greater extent than women.^{35,36} This inference is clouded however, by the presence of significant weight loss in male subjects (-3.42 kg) but not in female subjects (-0.88 kg).³⁵ As outlined above, perturbations in energy balance weaken any conclusions that may be drawn regarding the independent effects of hypobaric hypoxia on substrate utilization.

6. Rationale for how women may respond to altitude

Substrate utilization may vary between genders and across the menstrual cycle at sea level.^{53,70} As a result, women may respond to altitude differently than men and the response may be different in the two phases of the menstrual cycle.

Comparisons between genders and across the menstrual cycle have identified a number of mechanisms that may contribute to alterations in substrate metabolism. Differences across the menstrual cycle are primarily attributed to the effects of ovar-

ian hormones (estrogen and progesterone), whereas differences between genders can result from a number of factors in addition to ovarian hormones (Table 1).

Because the ovarian hormones play a major role in each comparison, this discussion will focus on the contribution of estrogen and progesterone to variations in substrate utilization.

Metabolic effects of estrogen and progesterone

Estrogen and progesterone potentially affect every aspect of substrate metabolism. Fluctuations in ovarian hormones have been linked to altered intake, storage, mobilization, cellular uptake and oxidation of carbohydrate and lipid, but the degree of these effects is unknown.^{2,14,24,45,53,60,71,73} Exogenous administration of ovarian hormones (e.g. in studies of oral contraceptive users or using animal models) results in significant metabolic changes^{48,55,70} However, with certain endogenous variations (e.g. across the menstrual cycle, between genders), the metabolic effects have proven more subtle.^{4,6,19,21,23,25,27,30,31,34,37,41,44,46,49,51-53,57,59,74,75}

Estrogen enhances the use of fat as a fuel, and estrogen and progesterone together may have actions sparing carbohydrate use.^{14,45} There is some evidence that the metabolism of protein and ketone bodies responds to ovarian hormones (differences in nitrogen excretion have been observed between the genders and across the menstrual cycle) but these effects require further investigation.^{19,24,49,74,75} Some potential actions of estrogen and progesterone to regulate substrate metabolism are shown in Table 1. Estrogen and progesterone have direct effects on liver, muscle and fat tissue, as well as indirect effects mediated by insulin, cortisol, growth hormone and plasma catecholamines (Table 2, Fig. 1).

To make an educated guess regarding the net result (e.g. increase or decrease in the rate of lipolysis) of the hormonal milieu, both the relative concentrations of estrogen and progesterone and their relationship with other regulatory factors must be considered. Because so many factors interact with estrogen and progesterone, it is an oversimplification to make predictions based solely on the actions of ovarian hormones. Still, estimating the net effect of different relative concentrations of estrogen and progesterone (for example: high levels of estrogen with or without progesterone) may offer insight into predicting how women will respond to high altitude. Figure 1 illustrates how the ovarian hormones could direct substrate trafficking based on their theoretical metabolic effects.

Variation in substrate utilization across the menstrual cycle

A woman's menstrual cycle is characterized by fluctuating levels of ovarian hormones (see chapter by LG Moore in this volume). It has been suggested that alterations in substrate metabolism are associated with the hormonal changes.⁵³ Variations in substrate selection may influence, and be influenced by, the response to an environmental factor such as hypoxia.

The literature on substrate utilization across the menstrual cycle, at rest and during exercise, is inconclusive. Interpretations are difficult because the techniques used to assess substrate use are imprecise (e.g. plasma substrate concentrations are used as an index of fuel oxidation). Even so, some trends are apparent (Table 3).

Studies in which plasma concentrations of metabolites (such as lactate, glucose and free fatty acids) are measured give conflicting results, sometimes indicating higher fat use in the luteal phase, but often showing no differences.^{6,37,52,59} In

Table 2

PREDICTED EFFECTS OF ESTROGEN AND PROGESTERONE ON METABOLISM	
LIPID	
<i>Estrogen</i>	<ul style="list-style-type: none"> * Decrease TG Lipase in liver * Increase synthesis and release of hepatic TG * Increase TG release from adipose * Increase adipose HS LPL activity * Decrease adipose LPL activity
<i>Progesterone</i>	<ul style="list-style-type: none"> * Increase LPL activity in liver and adipose * Decrease adipose lipolysis * Increase adipose lipogenesis * Partially reduces hepatic TG release caused by estrogen
PROPOSED NET EFFECT: Estrogen promotes lipid mobilization and use, progesterone promotes lipid storage	
CARBOHYDRATE	
<i>Estrogen and Progesterone</i>	<ul style="list-style-type: none"> * Increase substrate conversion to glycogen at liver * Decrease gluconeogenesis in liver * Decrease insulin binding capacity
<i>Progesterone</i>	<ul style="list-style-type: none"> * Decrease glucose uptake and oxidation by adipose tissue * Decrease glucose incorporation into fat by adipose tissue * Decrease peripheral insulin sensitivity * Increase insulin production by direct effects on the pancreas
PROPOSED NET EFFECT: Progesterone and estrogen inhibit carbohydrate mobilization and promote carbohydrate storage	
PROTEIN	
Progesterone	may be weakly catabolic
Estrogen	may be weakly anabolic
Metabolic actions of estrogen and progesterone as indicated by animal, oral contraceptive and in vitro studies (14,45,48,53,55,70). TG: Triglyceride HS LPL: Hormone-sensitive Lipoprotein Lipase LPL: Lipoprotein Lipase.	

several studies, respiratory exchange ratios are lower in the luteal phase, suggesting that women burn more fat and less carbohydrate, but this is also not a consistent finding.^{25,27,44,46} Presumably, increased fat use would be observed in the luteal phase because the high levels of estrogen enhance lipolysis while estrogen and progesterone inhibit carbohydrate use. However, in one study a positive correlation was found between estrogen level and carbohydrate use, which the authors attributed to increased insulin sensitivity.² Clearly, more work is needed.

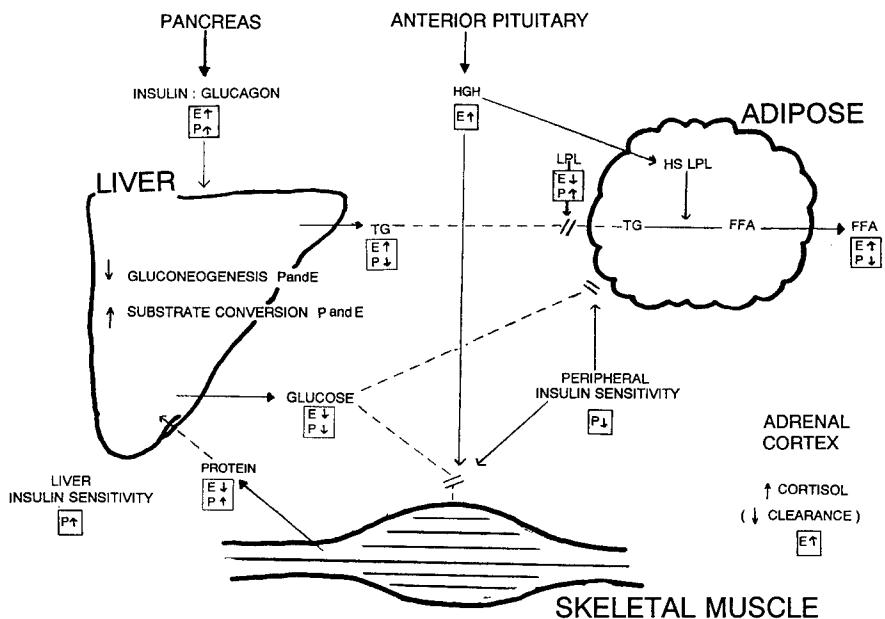


Figure 1

Although some studies report no difference when comparing the two cycle phases, the fact that differences tend to be in a consistent direction indicates a menstrual phase effect, albeit a subtle one. The possibility of fluctuations *within* a phase (e.g. early follicular vs. late follicular) should also be considered. The hormonal milieu can be drastically different between two timepoints within a single phase.

Table 3

Measurement	Luteal vs. Follicular	REF.	Women vs. Men	REF.
Respiratory Exchange Ratio During Exercise	L < F	27,44, 59	♀ < ♂	4,21, 74,75
Plasma Free Fatty Acids During Exercise	L = F	6,52,59	♀ > ♂ (sedentary) ♀ = ♂ (endurance trained)	4,23, 30,74, 75
Resting Muscle Glycogen Content	L = F, L > F	34	♀ = ♂	74,75
Exercise Muscle Glycogen Utilization	L = F	59	♀ < ♂	74
Glycogen Repletion Following Depletion	L > F	59	♀ = ♂ (55-60% CHO diet) ♀ < ♂ (75% CHO diet)	75
24-hour Nitrogen Excretion (Rest day)	L = F Rise in ML and MF Fall during ovulation	19,24, 31	♀ = ♂	24
24-hour Nitrogen Excretion (Exercise day)	ML > Menses	49	♀ < ♂	74,75

Some of the metabolic substrate differences reported from studies of the menstrual cycle and gender. The literature on most of these measures is sparse and the results and interpretations can be conflicting. No trend is stated unless at least one study has found a significant ($p < .05$) result. Phases of Menstrual Cycle (L: Luteal F: Follicular ML: Midluteal MF: Midfollicular) ♀: Women ♂: Men CHO: Carbohydrate

Methodological differences, environmental fluctuation, and the inherent biological variability among individuals preclude drawing firm conclusions from the available data.

Gender differences in substrate utilization

When not challenged by extreme environmental conditions, substrate utilization is not vastly different between men and women. Both genders rely predominantly on fat oxidation at rest, shift to increasing carbohydrate use during exercise, and burn progressively more carbohydrate as exercise intensity increases.⁷⁰ However, there is evidence for subtle gender differences in rest and exercise substrate metabolism, and some more pronounced dissimilarities in the metabolic response to exceptional situation such as hypoglycemia.^{1,52}

Methodological concerns need to be addressed when evaluating the literature. Plasma concentration of substrates or respiratory exchange ratios (RER) have been the typical methods used to monitor substrate utilization. In addition, attempts to match male and female subjects in terms of body composition or aerobic capacity have not been consistent. Level of training, body fat percentage, menstrual cycle status, etc. may be confounding factors. With these caveats in mind, trends should be interpreted warily.

At rest, some data suggest that men and women oxidize different percentages of fat and carbohydrate.^{41,60} However, if there is a difference, the direction is unclear. Studies in which resting RER values were measured indicate that, compared with men, women obtained a larger⁴¹ or a smaller⁶⁰ percent of energy from fat. Although gender differences are difficult to evaluate, these conflicting data may illustrate an interesting concept. It is conceivable that the determinants of basal fat metabolism are not the same between genders. For example, total fat mass may be better correlated with fat oxidation in men than in women.⁶⁰ This line of reasoning implies that variation among individuals may outweigh a blanket effect of gender.

During exercise, there is a trend indicating women get a greater percentage of energy from fat than men at the same relative submaximal workload, but this conclusion is not supported by every study.^{4,41,54,64,70,74,75} Endurance training has been reported to narrow any gender difference,⁷⁰ but trained women still oxidize more fat than men according to other studies.⁷⁴ Some researchers speculate that the difference in substrate selection is mainly attributable to the direct and indirect actions of female sex steroids. However, there are many other established gender differences that may be involved (Table 1).⁷⁰

7. Will women respond with fat not carbohydrate? A theoretical model

Although *in vitro*, animal, and oral contraceptive studies have begun to clarify the independent actions of estrogen and progesterone, the integrated effects of the ovarian hormones *in vivo* are difficult to predict. Their relative concentrations, interaction with other hormones, and the physiological state of the organism all modulate the magnitude and direction of any metabolic perturbation. To further complicate the issue, gender differences are clearly ascribable to more than the presence or absence of estrogen and progesterone; testosterone, body composition, muscle physiology, etc. all interact in complex and poorly understood ways.

Based on trends in the literature and the theoretical considerations outlined above, there is a credible rationale to expect that unlike men, women may not

respond to the stress of hypoxia by shifting substrate utilization in the direction of more carbohydrate and less fat. Both men and women increase sympathetic nervous activity in response to altitude, resulting in higher concentrations of the catecholamines epinephrine (increase upon acute exposure then back to sea level after about 7 days) and norepinephrine (steadily increasing over the course of 2-3 weeks). Rates of both glycogenolysis and lipolysis are enhanced by elevated levels of catecholamines. In male subjects, β -adrenergic blockade unexpectedly increased carbohydrate and decreased fat utilization at altitude (probably because lipolysis was downregulated even more than glycogenolysis in response to propanolol).^{67,68} In men, it may be that carbohydrate utilization is stimulated to a greater extent than fat utilization when subjects are exposed to hypobaric hypoxia. In women, estrogen and progesterone have direct and indirect (altered insulin secretion and sensitivity, levels of growth hormone, cortisol, etc.) effects to restrain carbohydrate oxidation and augment fat utilization. It is possible that the hormonal milieu in women will favor the predominance of fat use over carbohydrate utilization in response to hypobaric hypoxia.

To address this question, we recently studied 16 young women for 12 days in each phase of the menstrual cycle at sea level, and during one cycle phase at 4300 meters on Pikes Peak. A carefully controlled diet resulted in a mean weight loss of only 0.5 kg in these subjects after 12 days. Analyses of the isotopic data are ongoing but results so far suggest that, unlike men, women do not respond to high altitude by shifting substrate utilization toward more carbohydrate and less fat use. These data await complete analysis and a more complete picture is forthcoming.

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CHAPTER 4

WOMEN AT ALTITUDE: OVARIAN STEROID HORMONES, VOLUME REGULATORY HORMONES AND PLASMA VOLUME DURING ACCLIMATIZATION TO 4300M

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Introduction

Virtually no systematic study has been devoted to the influences of ovarian hormones on acclimatization to high altitude. This situation is true despite a long history of anecdotal reports attesting to sex differences in the incidence of severe altitude illnesses such as HAPE, and evidence that ovarian hormones influence key physiologic processes in high-altitude acclimatization.¹²

The hormonal fluctuations of the menstrual cycle are associated with hemodynamic changes and an increase in hormones which favor sodium and fluid retention. We recently completed studies of systemic and renal hemodynamics in 16 healthy women residing in Denver, CO (1600 m) during both their mid-follicular and mid-luteal phases.² In the luteal vs. follicular phase, cardiac output increased by nearly 18%, due to increased stroke volume, blood pressure fell by 8% and systemic vascular resistance decreased by 21%. Renal plasma flow and glomerular filtration rate increased during the luteal phase, while plasma volume was slightly, but not significantly elevated. These data are consistent with dilatation of both veins and arteri-

oles, and mimic the changes observed in early pregnancy. If the elevated hormones decreased vascular tone, activation of the renin-angiotensin-aldosterone axis (RAA) to favor retention of salt and water would have been required to maintain vascular pressures, a concept that is consistent with the large increase in plasma renin and aldosterone and the large decrease in atrial natriuretic factor that were present in the luteal vs. follicular phase.² A 36% increase in norepinephrine levels ($p < .06$) was also observed during the luteal phase of the menstrual cycle, which, in turn, may have contributed to activation of the RAA axis. The release of renin is stimulated by increase in catecholamines, as well as by decreases in renal filtered sodium load or perfusion pressure.^{2,5}

In men, the usual circulatory adaptations to altitude are decreases in plasma volume, a fall in cardiac output and increases in arterial pressure and resistance—changes which are probably mediated by the sympatho-adrenal system.¹⁰ In men at altitude that venous tone increases and plasma volume decreases.^{10,13} Data in women suggest that plasma volume shrinkage is reduced both in rate and magnitude⁴ but is maintained longer.^{4,14} However, the effect of menstrual phase is unknown. Because our previous work indicated that the luteal phase is associated with peripheral vasodilation and a stimulus towards water and sodium retention, we hypothesized that acclimatization to high altitude during the luteal phase would be associated with less decrement in plasma volume than that observed in women acclimatizing during the follicular phase (Fig. 1).

Experimental results on the vascular effects of ovarian steroid hormones are consistent with the clinical data on menstrual cycle effects presented above, but also illustrate the difficulties in predicting what effects the menstrual cycle may have upon the process of acclimatization to high altitude. Altitude exposure is associated with activation of the sympathetic nervous system, increase in circulating catecholamines, and a somewhat variable inhibition of the renin-angiotensin-aldosterone axis.^{5,10} Ovarian hormones interact with sympatho-adrenal mechanisms of vascular control in a number of ways, reviewed in the overview chapter by Dr. Lorna Moore,

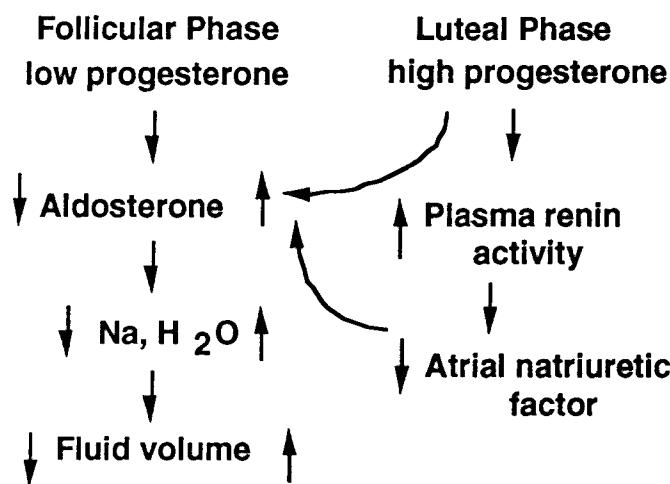


Figure 1 Model of the study hypothesis.

and it is clear that the combined actions of the two hormones are complex. Judging from the vasodilation observed in women during the luteal phase and in pregnancy, we think their combined effect is to relax both arteries and veins.

Previous investigations on altitude-associated alterations in the renin-angiotensin-aldosterone axis vary considerably in design and methodology. In general, it appears that at altitudes in excess of 3000 m, and in individuals not previously acclimatized to high altitude, aldosterone concentrations are consistently depressed, whereas plasma renin activity may be increased, decreased, or unchanged. These data are thoroughly reviewed in prior Hypoxia Symposia proceedings and elsewhere.^{3,5,6,9,11} The variability in plasma renin concentrations is likely due, in part, to variation in whether blood samples for analysis of volume regulatory hormones were obtained at rest, during exercise, at various times of day, and at various times during the acclimatization process. There are two differing interpretations of the literature on altitude-associated alteration in the RAA axis. The model elaborated by Hershel Raff suggests that there is dissociation between renin and aldosterone under hypoxic conditions and in certain disease states.⁶ An alternative view suggests that when sodium and fluid intake are controlled, and when samples collected for analysis of RAA-associated hormones are obtained under rigorous, standardized conditions (i.e. always at the same time of day, at rest etc.), no dissociation between renin and aldosterone is observed, and both are lowered during at least the early stages of acclimatization.⁹

The control of aldosterone synthesis and release is complex, being stimulated by renin (via renin's affects on the synthesis of angiotensin II), potassium ion and ACTH, and can be both directly and indirectly inhibited by atrial natriuretic factor (Fig. 2). Because aldosterone production is generally very closely linked with plasma renin activity, it is surprising that altitude exposure is often associated with low aldosterone relative to renin. Healthy acclimatization is associated with diuresis and a fall in plasma volume, thus one would expect both renin and aldosterone to decline during acclimatization. Alternatively, since altitude exposure is most certainly asso-

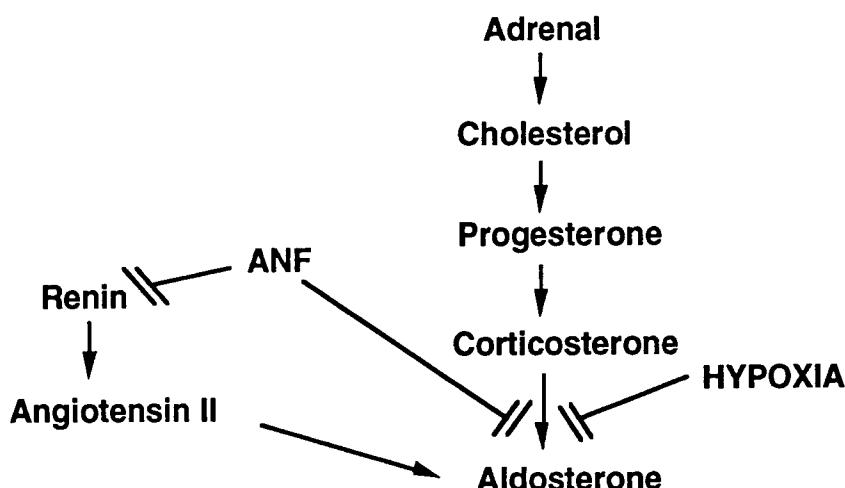


Figure 2 Model of the renin-angiotensin dissociation hypothesis.

ciated with increases in circulating catecholamines, an increase in renin and hence aldosterone might be expected, but is not observed. This suggests that the inhibitory stimulus of hypoxia can over-ride SNS activation of the RAA axis. Raff and colleagues have demonstrated that aldosterone conversion from corticosterone can be directly and markedly inhibited by physiologic reductions in PO_2 , due to inhibition of the mitochondrial cytochrome P-450 enzyme, aldosterone synthase (Fig. 2).^{1,7,8} Therefore, a mechanism exists to explain altitude-associated reduction in aldosterone, but the role of renin and the other hormones which influence aldosterone is unclear. Pregnenolone is the precursor for all mineralicorticoids, as well as the sex hormones. Since pregnenolone and its product, progesterone, are increased during the luteal phase of the menstrual cycle (Fig. 2), we hypothesized that the increased progesterone present during the luteal phase may provide a sufficient surfeit of substrate to over-ride the inhibitory effect of hypoxia on aldosterone production and favor retention of a normal profile of luteal phase production of aldosterone.

To test this hypothesis, as a part of a much larger study, a total of 21 women were admitted to the Aging Study Unit of the Palo Alto Veterans Administration Health System for a 12-day protocol which commenced on either the first day after menses began or the day after a luteinizing hormone (LH) surge was detected (by ovulation predictor kit). Following the cycle of initial study, the same women were admitted for a repeat 12 day study protocol in the opposite phase. Sixteen of these women were transported to Colorado within 24 hr. of detection of either menses or an LH surge, and studied for 12 days in the Maher Memorial Research Laboratory on top of Pikes Peak (Colorado, USA). High altitude studies took place a minimum of 4 weeks and a maximum of 10 weeks after completion of sea-level studies. All women were iron-supplemented between study periods after the completion of their first cycle phase study at sea-level. Blood volume and volume regulatory hormones were measured on day 12 at sea-level and on days 3 and 11 at high altitude. Serial measurements of ovarian steroid hormones were obtained throughout the studies, including the days when volume regulatory hormones and blood volume were measured.

Subjects

The 21 women studied at sea level averaged 23 ± 1 yr. of age, 167 ± 1 cm in height, 63.4 ± 1.7 kg in weight, were healthy and without any history of chronic disease. None of the women were endurance athletes (as determined by a screening VO_2 max of <60 ml/kg/min). Normal menstrual cycle length ranged from 26 to 34 days. All women had resided at sea level for 2 years or more, without any significant altitude exposure for more than a few days (e.g. weekend ski trips). Volunteers were interviewed and their medical records reviewed by qualified personnel to determine whether inclusion criteria were satisfied.

Statistics

The follicular and luteal phase groups were compared at high altitude using one-way analysis of variance. The change in a given variable from sea level to altitude was compared between groups using one-way analysis of variance. A two-way nested design was used to compare altitudes and cycle phase. Relationships between variables were assessed using correlation and regression techniques. Comparisons were considered statistically significant when the P value was <0.05 .

Methods

The dietary controls that were in place for these studies are reviewed in the chapters by Drs. Butterfield and Braun. From the perspective of volume regulation it is important to note that fluid intake was prescribed and monitored, and sodium and potassium intake were controlled. Red cell mass and plasma volume were measured using a modified CO rebreathing technique as previously described.¹⁴ Hemoglobin concentration was measured with an OSM (Radiometer) and hematocrit by the microhematocrit technique. All hormones were measured using radioimmunoassay. Intravenous catheters were placed a minimum of 45 minutes prior to blood collection. The catheters were placed and blood samples were collected from all women after they awoke in the morning, between 7:00 and 9:00 AM. Samples were drawn after a minimum of 30 minutes rest in a darkened, quiet room, were processed immediately, and stored at -70° until analysis. While measures of several other hormones, and of extracellular fluid and total body water were obtained, only measurements of blood and plasma volume, ovarian steroid hormones, and aldosterone and plasma renin activity have been processed to date, and the results and interpretations presented here are preliminary.

Cycle phase influences at sea level

There was no difference in plasma volume measured during the luteal vs. follicular phase within a given subject (n=13 women with hormonally verified follicular and luteal phase data). Plasma renin activity was 44±9% higher in these same women during the luteal vs. follicular phase. Consistent with the higher plasma renin concentrations, aldosterone was elevated by 40±11% in women studied in the luteal vs. follicular phase (p<.05). The serial measurements of estradiol and progesterone showed a normal pattern of cyclic change.

Influence of High altitude and cycle phase

While similar numbers of women theoretically arrived in the luteal and follicular phases of the cycle, serial serum ovarian hormone measurements indicated that 5 women were studied in the luteal phase and 11 in the follicular phase. The serial measurements of ovarian steroid hormones did not differ from those measured at sea-level. One woman in the luteal phase who was treated for AMS with Diamox was excluded from analysis of day 3 data.

By day 3 of residence at 4300 m, luteal phase women had a fall in plasma volume of -16±12%, and no change in red cell mass (+8±9%, p=NS vs. sea level). In the 11 subjects who acclimatized during the follicular phase, day 3 plasma volume was -16±3% relative to sea level and red cell mass did not change (-6±3%, p=NS). Taken together, all subjects had a decrease of -17±3% in plasma volume (p<.05 vs. sea level) and red cell mass was similar to sea level (-4±3%) at day 3 of altitude exposure. The change in blood volume was similar between the follicular and luteal phase women. Plasma renin concentrations were lower than values measured at sea-level in both groups of women (-50±10% follicular and -45±20% luteal, p<.05 vs. sea-level). Aldosterone concentrations on day 3 were also lower than values measured at sea-level in both groups of women, with a more pronounced fall in women who were measured in the luteal phase (-12±3% in luteal phase women and -4±1% in follicular phase women, p<.05).

By day 11 of high altitude residence, plasma volume was $-10\pm4\%$ lower than sea level values in follicular phase women ($p<.05$), having risen slightly (but not significantly) between days 3 and 11. Luteal phase women had day 11 plasma volumes that were $-13\pm8\%$ lower than sea-level, but due to the small sample size and variability of the measurements, plasma volume in these women no longer differed from the values measured at sea-level. Red cell mass increased $13\pm6\%$ in luteal phase women and by $4\pm4\%$ in follicular phase women, for an overall increase of $6\pm4\%$ in all women ($P=NS$ for any comparison). Aldosterone concentrations remained low in women who acclimatized in the follicular phase, and were still $15\pm1\%$ below values obtained at sea-level on day 11 at 4300 m ($p<.05$). In contrast, aldosterone concentrations were higher at day 11 than at day 3 in women who acclimatized during the luteal phase and did not differ from values measured at sea-level. Similarly, plasma renin activity remained depressed in follicular phase women at day 11 ($-15\pm14\%$), whereas values rose in luteal phase women and resembled those obtained at sea-level. There was thus a significant interaction between cycle phase and altitude exposure with respect to these volume regulatory hormones.

In both the sea-level and the high altitude phases of the study, changes in plasma renin activity and aldosterone were correlated within subjects, particularly during the luteal phase of the menstrual cycle, such that higher levels of plasma renin activity were associated with higher concentrations of aldosterone.

Summary

Our sea-level data confirm our prior observations in Denver (1600 m), and demonstrate activation of the RAA system during the luteal phase of the normal menstrual cycle, without change in plasma volume. Exposure to 4300 m altitude did not affect ovarian steroid hormone concentrations. The hypothesis that acclimatization during the luteal phase would be accompanied by less decrement in plasma volume, in plasma renin activity and aldosterone concentrations was not supported early in acclimatization (day 3). The data are less clear with respect to day 11, where luteal phase women had higher renin and aldosterone concentrations, and the changes in plasma volume were quite variable. Because sample size was small and the measures variable, further evaluation of menstrual cycle phase effects in the early stages of acclimatization is needed to fully evaluate the hypothesis. The hypothesis that increased progesterone concentrations may over-ride the hypoxia-associated inhibition of aldosterone was partially supported, since the clearest cycle phase-associated difference at high altitude was in the pattern of change in aldosterone and plasma renin. Luteal phase women reversed the altitude-associated decline in the concentrations of these hormones at some time point between day 3 and day 11 of acclimatization, with day 11 values comparable to those obtained at sea level. In contrast, follicular phase women had a fall in plasma renin and aldosterone concentrations which remained lower than at sea level on day 11. There was no hypoxia-associated dissociation between plasma renin activity and aldosterone in this study of healthy young women. The lack of dissociation between renin and aldosterone in this study might be due to inherent differences between men and women, since only men have been systematically studied in the past, but are far more likely to be due to the measurement conditions, i.e. samples were obtained from quiet, resting subjects whose dietary and fluid intakes were controlled, using a standardized protocol designed to minimize the effects of diurnal variation and environmental conditions (other than altitude).

Additional study over a longer period of acclimatization would be useful in evaluating whether the return of these hormones to sea-level values is associated with return of normal plasma and other fluid volumes. Samples are currently under analysis for concentrations of catecholamines, angiotensin II, atrial natriuretic factor, extracellular fluid volume and total body water. Such data should provide a more complete picture of fluid shifts in healthy young women at altitude and will also permit further evaluation of the contrast in the pattern of change in volume regulatory hormones in women acclimatizing in the luteal vs. follicular phase of the menstrual cycle.

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CHAPTER 5

WOMEN, EXERCISE, AND ACUTE MOUNTAIN SICKNESS

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Introduction

Acute mountain sickness (AMS) has been studied predominantly in men. However, due to physiological differences between genders (including, but not limited to, ovarian hormones), women may have different physiological responses to altitude than men. This may lead to a different incidence and severity of AMS in women. In this chapter we review the influence of the menstrual cycle, oral contraceptives (OCs), and exercise in women on susceptibility to AMS.

Incidence of AMS in Women and Men

Few researchers have compared the incidence and severity of AMS between women and men, and the results are contradictory. Honigman et al²⁶ studied 3,158 adults visiting moderate altitude (1900-2500 m) for recreation. Of 1,255 women included in that study, 28% developed AMS compared to 24% of the men ($p<0.01$). In another survey conducted at a higher altitude (~4000 m), Hackett et al²¹ studied 278 unacclimatized trekkers in Nepal and noted no gender differences in AMS susceptibility. Similarly, Maggiorini et al²⁸ studied 466 climbers (17% women) in the Swiss Alps (2800-5000 m). The incidence of AMS symptoms between men (53%) and women (57%) was not different. However, there was a significantly higher incidence of HAPE in men (13%) than in women (<1%). Forty-nine men, but only one woman, had to be air-rescued due to pulmonary edema.

Women at Altitude

The first research at altitude on women was most likely in 1913 when Mabel Fitzgerald rode on horseback through the Colorado Rockies and analyzed alveolar gases of altitude residents.¹² Fitzgerald studied 43 residents, men and women, aged 18 to 70 years at moderate altitudes. She found that P_{AO_2} and P_{ACO_2} were the same in men and women and that hemoglobin concentration was not uniformly higher at

altitude in women, as it was in men. It was 55 years until the next major investigation of women at altitude. In 1969 Hannon et al²⁴ examined altitude acclimatization in eight women (19-21 years) who spent the summer working and living on the summit of Pikes Peak (4,300 m). These and other studies form the basis for our limited knowledge of the responses of women to acute and prolonged high altitude exposure.

Menstrual Cycle

Hormonal differences between phases of the menstrual cycle in women may cause variations in response to altitude. The normal menstrual cycle is, on average, 28 days long and consists of two major phases: follicular and luteal. In the follicular stage, ovarian follicular growth occurs, and follicle stimulating hormone (FSH) and luteinizing hormone (LH) are secreted. The developing follicle secretes estrogen in response to FSH and LH which signals the hypothalamus to reduce secretion of FSH and LH. In the luteal phase, progesterone and estrogen are secreted by the corpus luteum. Progesterone also causes swelling and secretory development of the endometrium. Approximately 26 days into the normal menstrual cycle, estrogen and progesterone concentrations decrease sharply and menstruation occurs.

Menstrual Cycle and Ventilation

Of the two phases, the luteal phase may have the most effect on adjustment to altitude because during the luteal phase of the menstrual cycle progesterone levels are markedly elevated and progesterone is a potent ventilatory stimulant. For example, when synthetic progesterone is given as a supplement to men or women ventilation is markedly increased.³⁹ Increased ventilation upon exposure to altitude is said to be the body's first line of defense on exposure to altitude (Fig. 1).^{33,43} Thus, the increase in ventilation caused by high levels of progesterone during the luteal phase might be beneficial for high altitude acclimatization.

When women with complete hysterectomies were given either placebo, 1.25 mg estrogen, 20 mg progesterone, or both estrogen and progesterone together, ventilation was increased with progesterone and when given in combination with estrogen by $7\pm3\%$ and $12\pm6\%$, respectively.³⁹ Given in combination, the synthetic hormones also increased the hypoxic ventilatory response (HVR) but not when given alone ($p<0.05$). Therefore, if an augmented HVR is necessary for better adjustment to altitude, then women taking OCs may have an advantage when visiting high altitude.

Menstrual Cycle and Fluid Balance

Fluid retention is associated with AMS; it appears that individuals with a strong diuretic response suffer less from AMS than individuals who lack this response (Fig. 1).^{4,17,19,20,23,48} The changes in fluid regulating hormones with the normal menstrual cycle are still controversial; however, the results suggest higher levels of arginine vasopressin (AVP), plasma renin activity (PRA), and plasma aldosterone (ALD) during the luteal phase compared with the follicular phase.^{5,13,29} Other researchers found no significant differences in these hormones between phases of the menstrual cycle.^{10,35} It is clear, however, that if ovulation fails, there is no increase in these hormones.

Sundsfjord and Aakvaag⁴⁹ studied PRA and plasma renin substrate (PRS), as well as urinary ALD excretion (UAE), in 18 women. The women were divided into

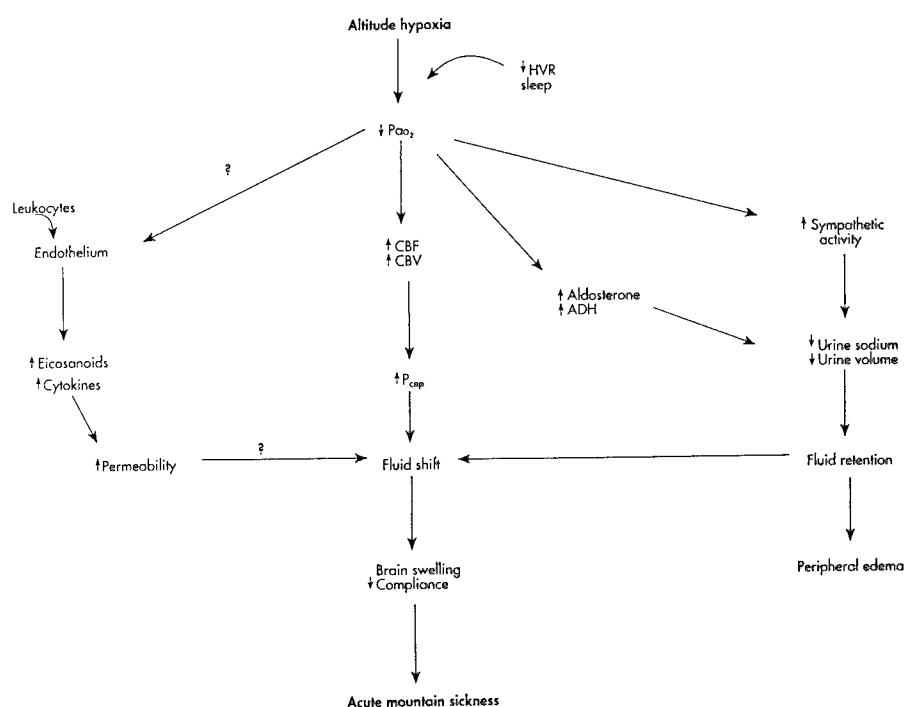


Figure 1 Proposed pathophysiology of acute mountain sickness. HVR, hypoxic ventilatory response; CBF, cerebral blood flow; CBV, cerebral blood volume; Pcap, capillary pressure; ADH, antidiuretic hormone. Reprinted from HACKETT P. H., R. C. ROACH. High-altitude medicine. In: Auerbach P. A., ed. Wilderness Medicine. St. Louis: Mosby, pp. 1-37, 1995.

either a luteal or a luteal-failure group according to their menstrual status. The luteal-failure group showed no significant difference in PRA between the first and second half of the menstrual cycle. However, the luteal group showed a significant increase in PRA during the luteal phase. No difference in PRS between menstrual cycle phases was noted for either group. The UAE rose significantly during the luteal phase in the luteal group, but did not change in the luteal-failure group.

Michelakis et al²⁹ measured PRA and ALD in six women throughout their menstrual cycles. Five of the 6 women had ovulatory cycles while one failed to ovulate and was considered anovulatory. In the ovulatory cycles both PRA and ALD increased during the luteal phase when progesterone levels were highest. The anovulatory subject had no change in either PRA or ALD throughout her cycle.

Forsling et al¹³ studied the variation in the AVP levels throughout the menstrual cycles of eight women. AVP levels were highest at the time of ovulation and lowest at the onset of menstruation. In a later study, Forsling and colleagues¹⁴ studied AVP levels in post-menopausal women given 2 mg/day of estradiol valerate and/or 10 mg/day of medroxy progesterone (MPA). Estradiol treatment alone resulted in significantly increased AVP levels, while MPA treatment alone had no effect on AVP levels. However, MPA in combination with estradiol treatment resulted in a gradual decrease in AVP levels. The combination treatment most closely mimics the physiology of the luteal phase. During the luteal phase of the menstrual cycle, both

progesterone and estrogen levels are high, thus it seems likely that the combination of these two hormones results in suppression of AVP.

Unlike the studies by Forsling,¹⁴ other researchers have reported no significant differences in AVP levels during the menstrual cycle; however, there are methodological differences that may account for the discrepancies. DeSouza and colleagues¹⁰ found no difference in AVP throughout the cycles of 16 female runners. Eight runners were amenorrheic and eight were eumenorrheic. No difference was found in resting AVP or PRA between the two menstrual cycle phases. However, ALD was significantly greater during the luteal phase in the eumenorrheic runners. The pre-exercise estrogen and progesterone levels for both the amenorrheic and eumenorrheic groups were similar. Thus, as is often the case with physically active women, especially runners, estrogen and progesterone levels can be lower than in their less active counterparts.

Punnonen and others³⁶ studied AVP changes during the menstrual cycles of 14 ovulatory women. AVP was not significantly different throughout the cycle. However AVP tended to increase at the time of ovulation when estrogen was highest and to fall when progesterone was rising. The failure to find significant differences in AVP levels may be due to not measuring AVP during menstruation when AVP levels are the lowest.¹³

The effect of menstrual status (eumenorrheic versus amenorrheic) on ALD, atrial natriuretic peptide (ANP), and PRA was further studied in a group of women, aged 18 to 37 years.¹⁰ Plasma ALD was found to be lower in the follicular phase than the luteal phase in the eumenorrheic women. In addition, ALD was higher in the eumenorrheic group versus the amenorrheic group during a submaximal exercise test. Before exercise, plasma ANP and osmolality were similar between menstrual cycle phases and between eumenorrheic and amenorrheic groups, but four minutes after exercise ANP was elevated similarly in all groups. Plasma volume changes were similar between groups and no significant relationships existed between plasma ANP or PRA. Progesterone and ALD were positively correlated during the luteal phase of the menstrual cycle. In summary, when a woman travels to altitude during her luteal phase it is unknown whether the high levels of progesterone would be of benefit due to increasing ventilation, or whether they would be detrimental by causing fluid retention and thus rendering her more susceptible to AMS.

Menstrual Cycle and Exercise Performance

Exercise performance may be affected by phase of the menstrual cycle. Schoene et al⁴⁷ examined exercise performance between menstrual cycle phases in highly trained eumenorrheic women runners, non-trained eumenorrheic women, and highly trained amenorrheic women runners, aged 17 to 37 years. They compared the effects of menstrual cycle phase in the eumenorrheic women and the effects of training status in all women. They measured the HVR, hypercapnic ventilatory response (HCVR), exercise $\dot{V}_E/\dot{V}O_2$ and $\dot{V}O_{2\max}$. The eumenorrheic women had significant increases in resting ventilation and HVR during the luteal phase, with a higher HVR in the eumenorrheic athletes compared to the non-athletes. The amenorrheic group had no differences in HVR from the highly trained eumenorrheic women. The HCVR was higher in the luteal phase for the eumenorrheic athletes but not for the non-athletes. Also in the eumenorrheic women, the non-athletes had a greater exercise performance during the follicular phase versus the luteal phase, while there was

no change in performance in the eumenorrheic athletes. The $\dot{V}_E/\dot{V}O_2$ was greater in the luteal phase for all eumenorrheic women during the entire exercise protocol. The mechanism of the increase in ventilation during the luteal phase is not known.

The possible relationship between increased ventilatory response and reduced exercise performance has also been examined.⁶ Ten untrained men (age 27 years) were given ten milligrams medroxyprogesterone acetate (MPA) or placebo before an exercise test in a double-blind study. Each subject performed a $\dot{V}O_{2\max}$ test under the influence of MPA or placebo. HCVR was unchanged, resting P_aCO_2 was reduced, and resting pH was increased with MPA administration. Exercise blood gases were similar between groups. Blood lactate increased and bicarbonate decreased more in the MPA group than the control group ($p<0.05$). There was no overall effect on cardiovascular function with MPA, and no difference between MPA and control in $\dot{V}O_2$ for a given workload. Maximal exercise performance as measured by $\dot{V}O_{2\max}$, maximum workload, and perceived exertion did not change with MPA treatment. During submaximal performance, \dot{V}_E increased only at 33 and 50 percent $\dot{V}O_{2\max}$. When related to absolute carbon dioxide output rather than relative oxygen uptake, \dot{V}_E with MPA was increased at all workloads. The authors suggested there may be a greater effect of MPA during endurance exercise and that their results were similar to studies of women in their luteal phase. However, MPA has 15 times the progestational activity of naturally occurring progesterone and may not cause the same hormonal elevation of the *in vivo* control of ventilation.

Regensteiner et al⁴⁰ used mild and moderate exercise to manipulate metabolic rate and measured HVR and HCVR. They studied 12 women (23 to 40 years) in their follicular phase and 13 men (22 to 35 years) who were recreational athletes. End tidal PCO_2 and PO_2 , \dot{V}_E , SaO_2 , heart rate and tidal volume were measured. Mild exercise consisted of leg lifts that increased resting $\dot{V}O_2$ by 25%. Moderate exercise increased $\dot{V}O_2$ to approximately four times resting and consisted of cycling at 37 Watts for women and 49 Watts for men on a cycle ergometer. Women had greater ventilatory equivalents for O_2 and CO_2 and tended to have lower end tidal PCO_2 in mild and moderate exercise. Therefore, women had greater alveolar ventilation which could not be accounted for by HVR or HCVR. Resting HVR and HCVR were similar between genders. However, during mild exercise, HVR was greater in men suggesting that they have greater sensitivity to mild changes in metabolic rate.

Oral Contraceptives

Studying women taking OCs offers an opportunity to look at responses to altitude with control over hormonal fluctuations that occur during the normal menstrual cycle. It is unknown whether OCs would make women more or less susceptible to AMS. With oral contraceptive use, the levels of progesterone and estrogen are kept at an elevated level in relation to the follicular phase, and therefore the hormonal status is similar to the luteal phase and may have the risks and benefits during altitude exposure that are associated with the luteal phase of the menstrual cycle. Oral contraceptives consist of synthetic progesterone and estrogen and are taken to prevent ovulation or to regulate menstruation. The levels of estrogen and progesterone are kept constant throughout the cycle with monophasic OCs, but the hormone levels increase progressively with biphasic and triphasic OCs. The presence of these synthetic progestin and estrogens prevents the secretion of FSH and LH releasing factors from the hypothalamus which normally act on the pituitary

to release FSH and LH.⁴² Because of the absence of these gonadotropin hormones, follicular growth and maturation, and ovulation are prevented,⁴² ovarian steroid production is inhibited, and the synthetic steroids now maintain the uterus.⁴² As occurs with the natural withdrawal of progesterone and estrogen in the last week of a eumenorrheic woman's menstrual cycle, the cessation of OCs in the last seven days of a 28 day cycle causes menstruation to occur.⁴²

A concern when studying women who are taking OCs at altitude is that there are many types of OCs and the research comparing the potencies of different OCs is conflicting. One test of potency between OCs is the delay of menses test.⁵¹ In this test, a set of OCs is taken every day. If breakthrough bleeding occurs before the last of the OCs are taken, the test is negative. If no bleeding occurs while the OCs are taken, the test is positive. Swyer⁵¹ used the delay of menses test on women (< 38 years) with normal menstrual cycles taking ethinyl estradiol, a synthetic estrogen, with either norethindrone, norethindrone acetate, ethynodiol diacetate or norgestrel. They found that norgestrel was two to three times more potent than norethindrone while norethindrone was two times more potent than norethindrone acetate and ethynodiol diacetate in the delay of menses test. Dorflinger¹¹ reviewed several studies on potencies of different OCs and concluded that delay of menses data indicated that norethindrone, norethindrone acetate, and ethynodiol diacetate are equivalent in potency and norgestrel is five to ten times and levonorgestrel is ten to twenty times the potency of norethindrone.

The elevated progesterone levels with oral contraceptive administration may result in higher resting ventilation. The potential effects of oral contraceptive use on ventilation were studied in twelve women aged 21 to 30 years.³² Vital capacity, tidal volume, resting \dot{V}_E , resting $\dot{V}O_2$, forced vital capacity in one second, midmaximal expiratory flow, and $\dot{V}O_{2\max}$ were measured prior to starting OCs, and then three and six months after starting OCs. Resting tidal volume increased after oral contraceptive treatment ($p<0.01$). Resting minute ventilation increased at three months of treatment versus before and decreased from three to six months, although these changes were not significant. Exercise ventilation increased over time with use of OCs.

Oral contraceptives may also alter fluid regulating hormones which could contribute to increased or decreased susceptibility to AMS. Huisveld et al²⁷ studied the effects of OCs and exercise on the renin-angiotensin system in 20 highly trained athletes (10 OC and 10 non-OC) compared to 24 sedentary females (13 OC and 11 non-OC). Women on OCs had suppressed renin angiotensin activity as measured by lower pro-renin and active renin concentrations, but higher renin substrate concentrations. This suppressive effect of OCs on the renin-angiotensin system was potentiated with exercise, suggesting that OCs may provide a protection from development of AMS by preventing fluid retention. However, another study showed no significant differences in renin activity between women using OCs compared to renin activity in days seven to 12 of a normal menstrual cycle in women not using OCs.⁹

Other effects of OCs include increased resting human growth hormone levels, decreased resting blood glucose, increased free fatty acid concentration during mild exercise,⁷ increased exercise human growth hormone, and increased reliance on fat and reduced carbohydrate oxidation during prolonged exercise. It is unknown whether these actions of OCs contribute to AMS.

Exercise and AMS

Rapid rate of ascent to altitude increases the severity of AMS.²² Hackett¹⁶ found that climbers taking less than four days to reach a 4,300 m base camp at Mt. McKinley were more likely to get ill than subjects taking five to 10 days. In this case, the rate of ascent was increased by increasing physical exertion and resulted in greater incidence of AMS. Our personal observations, along with anecdotal reports by others,^{15,34,38} suggest that overexertion may be related to the development of AMS. Also, the incidence of AMS is generally lower when subjects passively ascend to high altitude (as in an altitude chamber or by helicopter ascent on mountains) compared to when they exercise to gain altitude.³⁷ Thus, although prior physical fitness is unrelated to AMS susceptibility,^{18,25,26} exertion during ascent may increase the severity of AMS.

Exercise and Ventilation at High Altitude

It is well established that exercise at altitude decreases SaO_2 .^{8,46,50} West et al⁵⁵ reported that six experienced male climbers, aged 23 to 50 years, had reduced SaO_2 from rest to increasing workloads on a cycle ergometer, along with an increased alveolar-arterial oxygen difference at 5,791 m. This suggests that there are diffusion limitations in the lung resulting in a continued fall in the partial pressure of oxygen in mixed venous blood (P_vO_2) and a desaturation of arterial blood.^{52,54,55} In young men (21 to 31 years) studied in Operation Everest II, whose fitness ranged from trained to untrained, the alveolar-arterial oxygen difference increased and P_aO_2 was reduced with exercise.⁵⁰ Resting and submaximal cardiac output was maintained and P_vO_2 was reduced. The reduction in P_vO_2 and increase in arteriovenous difference was linearly related to $\dot{\text{V}}\text{O}_2$. This suggests that at a given submaximal $\dot{\text{V}}\text{O}_2$ at altitude, a given level of $\dot{\text{V}}\text{O}_2$ was achieved by reducing P_vO_2 instead of increasing cardiac output.^{50,53} If exercise does increase AMS symptoms, enhanced arterial oxygen desaturation and decreased P_vO_2 may be among the initiating events.

Ventilation is linked to arterial oxygen desaturation. Schoene et al⁴⁴ found a significant negative correlation between HVR and arterial oxygen desaturation at high altitude. Subjects with a brisk HVR were able to reach and sleep at higher altitudes than subjects with a low HVR. They concluded that subjects with a low HVR have a lower P_aO_2 and a higher PaCO_2 , which shifts the oxyhemoglobin dissociation curve to the right. Under these conditions, hypoxic exercise would facilitate unloading of oxygen from hemoglobin to the tissues, and limit loading of oxygen at the lungs, resulting in decreased SaO_2 .

Exercise and Fluid Balance at High Altitude

Exercise at any elevation affects fluid balance as does hypoxia without exercise. Therefore, it is important to understand the dynamics of fluid balance in humans when exercising at high altitudes.

Exercise at sea level increases AVP, PRA, and ALD secretion¹ which could cause fluid retention. During exercise over five days at low altitude, Milledge et al³¹ found that five men (23 to 48 years) had increased PRA and ALD activity at the end of every day, with peak values reached on the second or third days. Increased PRA and ALD activity may have directly caused retention of sodium and caused slight leg

edema.

In 18 male mountaineers, Bärtsch et al² found that ALD and AVP levels were greater before and after exercise in subjects with AMS than in subjects without AMS, and suggest that the sodium and fluid-retaining effects of the ALD and AVP responses override the renal effects of ANP in AMS.² In another study by the same group, fluid homeostasis was examined in 15 healthy mountaineers on a controlled ascent to 4,559 m.³ PRA, ALD, AVP, and ANP did not change at rest in subjects without AMS. Subjects with AMS had significant weight gain and increased levels of ANP. The positive correlation of ANP and the increase in cross-sectional area of the right atrium,³ and a decrease in hematocrit suggest that the increase in ANP in AMS may be secondary to fluid retention and an increase in central blood volume.⁴

In addition, nine male soldiers who performed submaximal exercise at sea level, on acute exposure to 4,300 m (after less than two hours at altitude), and during chronic exposure (after two weeks at 4,300 m) had increased levels of ANP while exercising only on acute exposure to altitude, but not while exercising at sea level or during chronic exposure to altitude ($p<0.05$).⁴¹ From these data it appears that the ANP response changes during acclimatization to altitude possibly because of decreases in cardiac output and stroke volume which would reduce atrial stretch and therefore inhibit ANP release. Thus, ANP may increase in subjects secondary to exercise but independent of AMS, and acclimatization to altitude may lead to a reduction in ANP levels. In summary, hypoxia-induced and/or exercise-induced increases in ALD, PRA, AVP and ANP may explain sodium and fluid retention in subjects developing AMS. Further studies are needed to determine the cause and effect relationships between hypoxia, exercise and the hormonal regulation of fluid balance at high altitudes.

Fitness and AMS

Success at altitude is difficult to predict. For example, some successful climbers have augmented HVR upon arrival at altitude while others have blunted hypoxic ventilatory responses.⁴⁵ If HVR is critical for successful climbing, then motivation or other factors may lead to the success of the latter group.⁴⁵ Sea-level $\dot{V}O_{2\max}$ does not seem to predict climbing success, likely because climbing is usually a prolonged submaximal activity.⁴⁵ Some have even suggested that a high level of training may be inversely related to climbing success at very high altitudes. For example, in Operation Everest II, two of the most highly trained subjects were removed from the chamber before the end of the study, suggesting more trained subjects may be less tolerant of extreme altitude. Milledge et al³⁰ studied fitness and AMS in 17 men aged 23 to 55 years. No correlation was found between fitness and AMS. Thus, fitness at sea level does not predict susceptibility to AMS, and very fit individuals may perform suboptimally at very high altitudes. The limited studies on women exercising in hypoxia give no reason to suspect significant differences from men in the relationship of sea level fitness to susceptibility to AMS.

Summary

Though it is tempting to speculate about significant differences between men and women acutely exposed to high altitudes based on the influences of the ovarian hormones, data available to date support only minimal differences in physiologic

responses, and essentially the same susceptibility to AMS. Further studies are currently underway to resolve the roles of gender, the menstrual cycle and oral contraceptives in physiological responses to both acute and prolonged hypoxia.

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CHAPTER 6

HYPOXIC LIFE AT THE BOTTOM OF THE SEA

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Sunlight at noon turns quickly to dusk as the submersible *Alvin* sinks below the ocean surface. Shafts of light that penetrate the water soon yield to the transparent darkness of the great ocean depths. The descent continues in the blackest of midnights, lit only by splashes of luminescent light from the planktonic organisms disturbed by the submarine's path through the water. Over the two and a half years during which I piloted *Alvin*, that quick darkening became oddly familiar, the comforting prelude to a long day of wonder. *Alvin* can take as long as two hours to reach the bottom on a typical dive of 11,000 feet. (Much of the ocean, of course, is far deeper, as deep as 35,000 feet, well beyond the sub's reach.) Once on the seafloor, even the fiercest lights won't penetrate very far. But in the sub's spotlight you can glimpse a minute patch of the largest, most inaccessible, and least understood ecosystem on our planet. Here you are likely to encounter fantastic creatures—single-celled protozoans of grapefruit proportions, giant stalk-borne tunicates looking like paper grocery bags on sticks, herds of rolling sea cucumbers congregating on the tan muds of vast soft-sediment plains. It is the biology of these and other, even more intriguing deep-sea animals that particularly interests me.

Our ignorance of this underwater world is almost unfathomable. In 1968, when *Apollo 8* brought back the first color images of Earth as it looks from space, we were shown a blue planet, with seas covering more than 70 percent of its surface. An alien scientist trying to understand Earth, it's often said, would name our planet Water and focus on the oceans.

Why then are our oceans terra incognita? Why has only a pitiful 1 percent of the seafloor been mapped? One reason is that support for exploring the deep oceans is burdened by historical misconceptions. Early-nineteenth-century scientists convinced themselves that the deep seas were not worth bothering about because nothing lived below 1,800 feet. Extreme cold, darkness, and tremendous pressure were thought inimical to life. This notion of an "azoic zone" was challenged in the late nineteenth century when ocean scientists—using surface vessels and dredging operations—scooped up vast quantities of organisms from deep water. But the legacy of the azoic zone is dying hard. The abyss, as far as most people are concerned, is a biological desert, a flat, unchanging expanse of sediment stretching drearily from one continent to another.

In the past 15 years, however, we've been shown again and again just how wrong this view is. The abyss is not an unchanging desert, not universally cold and dark, and certainly not uniformly flat. In the Pacific alone there are probably over a million underwater volcanoes. Linear mountain chains, 46,000 miles long in all, bisect

the ocean basins and girdle the globe like seams on a softball; we know now that the midocean ridges (as the mountain ranges are called) hold a key to understanding how Earth's crust is formed and, possibly, where life on our planet began.

Geologists know that the planet's underwater surfaces are constantly being resculpted. Those submarine mountain ranges, for example, mark the boundaries of the great crustal plates that form the seafloor. New seafloor is being generated here as the plates slowly spread apart (at the rate of some four inches a year, about the speed at which a fingernail grows) and molten volcanic rock wells up from Earth's underlying mantle. In an endless cycle, underwater earthquakes fracture the seafloor and lava erupts and fills in the fissures; earthquakes later rip these lavas too, creating new fissures.

Submersibles have barely explored a few tens of miles of this 46,000-mile system. Some of those miles I have logged myself at *Alvin*'s helm. The ridges are like highways, tarred black with volcanic lavas that have hardened into basalt. They do not rise to a crest at their midline; instead, the seafloor typically drops down 60 to 150 feet to form a valley that might be 300 feet or more in width. The freshest lavas from the most recent eruptions are found in these rift valleys. You can tell they are fresh because they still carry a rind of brittle, shiny glass—they haven't had time to weather or accumulate a veil of dusty sediment. The tortured terrain in the valley is filled with pits, caverns, tall pillars, and rubble. Lavas frozen in motion form pillows, whirlpools and ripples, drapes and lobes. It is a submarine landscape of stark beauty.

I have sometimes found fissures in these valleys large enough to descend into with the submersible. The walls of a fissure can be so sheer that the seafloor looks as if it had been sliced and wedged apart by a giant knife. I have driven deep into a fissure where the walls narrowed to within a yard of *Alvin*'s side windows. In front of me I could see the seam where the walls joined. I was at the very place where the planet's crustal plates were inexorably moving apart. I knew that hundreds of microearthquakes were being reported in the area—evidence that the plates were spreading—and I half-expected the fissure to open up and engulf me. I sought no excuse to loiter there.

In places where the seafloor is actively spreading, seawater percolates through cracks in the crust; as it seeps downward it is heated, and it exchanges chemicals and minerals with the molten rock underneath. This superheated mineral water—it can reach temperatures of 660 degrees or more—exits up through the seafloor in submarine hot springs called black smokers. Dissolved minerals in the hot, venting water precipitate as it mixes with frigid ocean, producing what look like plumes of turbulent black smoke. Cameras sensitive to extremely low levels of light show that an eerie glow is emitted by the hot water as it exits from the seafloor—a geothermal light source where no sunlight exists.

Where the subsurface rock is cooler, vent water flows out of the seafloor as diffuse plumes; with temperatures ranging from just above freezing to 100 degrees, these plumes are more hospitable to life than those at the black smokers (though even at some smokers swarms of shrimp somehow thrive without getting fried). Vent water is enriched with numerous compounds, including hydrogen sulfide, with its ripe odor of rotten eggs; a sample bottle opened in the laboratory can clear the room in seconds. Since the late 1970s microbiologists have discovered a variety of bacteria that thrive on the sulfide. These chemosynthetic bacteria use the sulfide's chem-

ical energy to produce organic carbon compounds in much the same way that plants, including plankton, use energy from light for photosynthesis. Occasionally blooms of chemosynthetic bacteria become so dense they create a whiteout, a patchy bacterial blizzard, within the ridge valley.

The implications of these findings are stunning; they suggest that hydrothermal vents support life in the absence of sunlight—without the photosynthesizing plankton that provide most sea creatures with food. In other words, vents provide their organisms with an alternative way to make a living. That's led some researchers to suspect that deep-sea vents may have been the crucible where life originated on this planet. Pillow basalts dating back nearly 4 billion years have been found in China and Australia. So it's possible that midocean ridges have existed for most of the history of our 4.5 billion-year-old Earth.

But while the question of life's origins at the vents may be controversial, there can be no doubt that life has adapted to the vent environment with aplomb. Entire communities of invertebrates—tube worms, bristle worms, clams, mussels, shrimp, and other small crustaceans—reside at the cooler vents, all of them ultimately dependent on chemosynthetic bacteria. Some organisms, like bristle worms and limpets, simply graze on the bacteria; others, like crabs, feed on the bacteria eaters. But in the most fascinating cases the invertebrates have formed symbiotic relationships with the bacteria. The invertebrates houses the bacteria and provides the chemicals required for chemosynthesis. In exchange the bacteria give up organic carbon compounds to the host so that the host expends little or no energy gathering its own food.

The classic example of this type of symbiosis is found in a remarkable tube worm that reaches several feet in length and is found only at hydrothermal vents along the submarine mountain ranges off the western coasts of Mexico and South America. In the Galápagos rift, for example, at a site called the Rose Garden, you can see hundreds of long white tubes, closely spaced, each displaying a brilliant blood red plume. There is absolutely nothing on this planet to compare with these thickets of animals. Alive, on the seafloor, they are beautiful. But when they are brought to the surface and extracted from their tubes, their long, flaccid trunks reveal a very odd story. The worms have no mouth, no gut, not even a vestige of a digestive system. They simply extend their gill-like red plume into warm vent fluids to absorb sulfide, carbon dioxide, and other compounds. These compounds are then delivered to an organ filled with chemosynthetic bacteria. The bacteria return the favor by providing food that their hosts have no other means of obtaining.

New and fascinating deep-sea species like these are being found all the time. In fact, far from being azoic, the warm-water vents resemble lush oases, as teeming with life as shallow-water coral reefs and salt marshes—but life with a very odd twist. At the Galápagos site pink fishes named bythitids actually live head down and tail up in the mouths of vents. What allows all these organisms to thrive in environments with such steep gradients of temperature and in waters full of compounds, such as iron and copper, that are ordinarily toxic at high concentrations? We don't know—life here is a mystery.

Away from the vents, in the great ocean plains, life is much less dramatic and often scaled down to minute proportions—threadlike worms, tiny snails, delicate, transparent clams. Yet the diversity of animals in the cold abyssal muds, it now appears, may rival the celebrated biodiversity of the tropical rain forests. Little is

known of this fauna, yet the healthy functioning of these benthic communities must be critical to the balance of the world's oceans when the known communities are multiplied by the unimaginably vast surface of the seafloor.

What still seems so remarkable is that 15 years ago we barely knew that most of these animals existed. We are mere novices at understanding the seafloor environment. Our forays into the deep sea seem lilliputian when compared with the space exploration program, and by NASA standards the resources of ocean scientists look almost meager. In 1992, for example, the government budget for oceanography research was roughly \$600 million. NASA spent \$8.5 billion on research and development that year—\$2 billion of it on the space station alone. As a consequence we actually know more about the surface of Mars and Venus, and probably more about the dark side of the moon, than we know about the topography of our own seafloor. Ocean scientists must stand on long lines to get their limited share of funds and ship time so that they can press on with their experiments. And as funding becomes tighter, as it has for most branches of science, the next generation of oceanographers may finish training only to find that research opportunities have become frozen or are decreasing.

What is particularly frustrating is that this funding crunch has coincided with a time of tremendous technological innovation that should allow ocean scientists to study the seafloor in novel ways. Technically it should soon be possible to sit in a regional control center and supervise robotic operations at a remote, offshore study site, or to turn on a desk computer and call up real-time images of a seafloor experiment over an acoustic modem-satellite link.

Over the past quarter of a century, thanks largely to *Alvin*, the United States has been the leader in deep-sea exploration. But we may be seeing our lead slipping away. Even as funding becomes harder to secure each year and *Alvin* is increasingly threatened with mothballs, other nations, especially Japan, are putting deep-ocean research at the top of their national basic science agenda. Yet surely the need to understand our fragile blue planet is greater than ever. In an era calling for global environmental awareness, isn't it time we became more aware of the watery environment that makes up three-quarters of our home?

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CHAPTER 7

BIOLOGICAL AND TECHNICAL ISSUES IN MEASURING PO₂ IN SPECIFIC SUBCELLULAR SPACES

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Abstract

Oxygen is required for normal rates of mitochondrial ATP production and thus for normal muscle activity. Isolated mitochondria exhibit a very high affinity for oxygen, sometimes more than two orders of magnitude higher than venous PO₂. Thus, the complete understanding of oxidative metabolism, particularly when one would normally consider oxygen availability to be limited, requires accurate measurement of PO₂ in each of the subcellular compartments from the vascular space to the inner mitochondrial matrix. In this series of presentations, we will hear about several different measurement methodologies and how the results of these measurements bear on our understanding of the biology of oxidative metabolism.

Overview

Oxygen is one of the few absolute requirements for the sustenance of mammalian life on earth. In the production of energy, particularly of energy required for the contractile activity of actomyosin ATPase, it is required that muscle produce enough ATP to allow for normal contractile activity. Although much is known about the role of oxygen in metabolism, accurate measurement of oxygen content, particularly in subcellular spaces, is quite difficult. Often, the best that can be done is to make an estimate of the degree of oxygenation. In this symposium, we will address the issues involved in making some of these measurements and in the integration between these measurements and metabolism as it pertains to muscle activity.

Exercise occurs via the contractile activity of skeletal muscle. The biochemical energy which drives the activity of myosin and actin is provided by the free energy contained in the terminal phosphate bond of ATP^{4,20}. The dominant biochemical process by which skeletal muscle produces ATP is mitochondrial oxidative phosphorylation³⁶. Thus, oxygen plays the dominant role in the metabolic processes which permit physical exercise in man.

In normally functioning skeletal muscle, carbohydrates and fats undergo a complex series of chemical reactions so that ultimately they are oxidized to their end products of CO₂ + H₂O with the transduction of this chemical energy into the bond

energy in the terminal phosphate ester bond of ATP (Fig. 1). This ATP is used by myosin ATPase to allow contractile activity. It is clear that these reactions require oxygen and that in sufficiently severe hypoxia, their rate would be attenuated or stopped altogether.

In the sections to follow, I would like to put oxygen availability into some relevant metabolic contexts. In so doing, it would be useful to keep foremost in mind the possible consequences to each of these metabolic systems of any oxygen unavailability.

Glycolysis

If you open any biochemistry textbook⁴⁸, you will find a chapter entitled glycolysis. The overall structure of this chapter will be to take the student sequentially from the beginning to the end of this metabolic pathway. The first step to be studied usually will be the reaction catalyzed by glycogen phosphorylase and the last step to be studied usually will be pyruvate dehydrogenase, in the context of this enzyme providing an acetyl group for entry into the citric acid cycle via the condensation reaction with oxaloacetate to produce citrate. If you examine each of these enzyme catalyzed steps carefully, you will see that none of these reactions require the participation of oxygen. This fact provides the framework for treating this metabolic pathway as an *anaerobic* pathway¹⁶, meaning that flux through this pathway can occur when no oxygen is present.

The pyruvate produced by the breakdown of glycogen (glycogenolysis) does not only provide acetyl units for entry into the citric acid cycle. Pyruvate is also the only precursor of lactate, the three carbon unit that is ubiquitous in the literature of

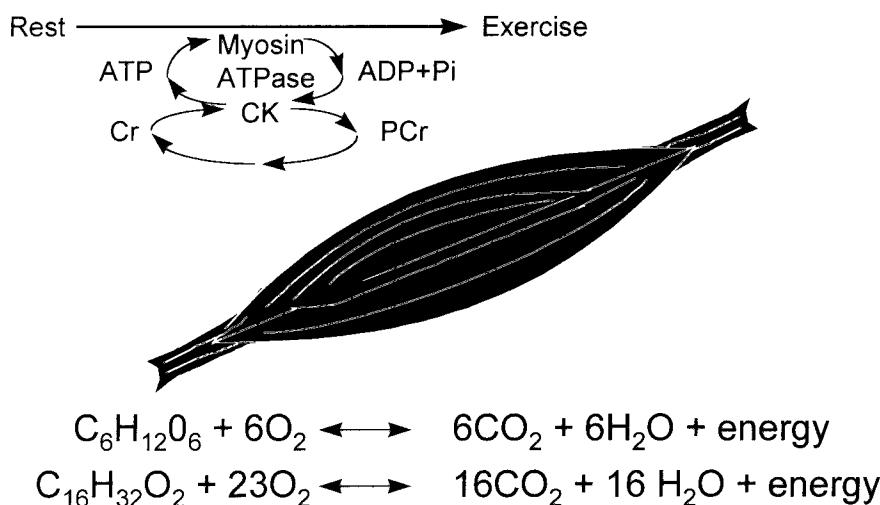


Figure 1 A model of the interaction between the action of myosin ATPase to use the chemical bond energy inherent in the terminal ester bond of ATP to provide the energy required for contractile activity and the action of mitochondrial oxidative phosphorylation of carbohydrates and fats to transduce the carbon-carbon bond energies of food into the chemical energy in the terminal ester bond of ATP. The net reactions of carbohydrate and fat oxidation are described stoichiometrically.

skeletal muscle physiology particularly as it pertains to exercise^{12,27,29}. Thus, it is common to consider an increase in the net production or accumulation of lactate as an index of anaerobiosis. It is well known that net lactate accumulation increases exponentially as a function of increasing physical exercise intensity⁴⁴. By very careful examination of the relationship between lactate concentration, either in muscle or blood, and exercise intensity, it is possible to identify an exercise intensity above which the concentration of lactate begins to increase so rapidly that it resembles a discontinuous function. A term, the *anaerobic threshold*, has been coined⁵³ to describe this point. As an individual increases exercise intensity, such as during a graded ramp-type exercise protocol, two things begin to occur and increase very rapidly at about the same point: lactate accumulation and rate of ventilation (both breathing frequency and total minute ventilation).

There are several problem with this sort of logic. One is that this discontinuity of ventilation occurs in human patients with McArdle's syndrome, in whom lactate cannot be produced¹³. But the more troubling problem is that an absence of oxygen, either hypoxia or frank anoxia, is not required for the production and accumulation of lactate^{6,28}. Thus, the general concept that either hypoxia or anoxia are required for glycolytic flux to increase to the point where lactate accumulates is almost surely incorrect⁵⁹.

A related problem with the relationship between hypoxia or anoxia and glycolytic flux involves the overall relationship between oxygen availability and lactate accumulation. Several things are known about this condition. The rate of lactate efflux is higher from fatigued muscle^{3,26}. Hypoxic muscle depletes its glycogen and makes lactate^{16,19}. The accumulation of lactate is at least closely associated with, if not the cause of, the fall in muscle pH that accompanies hypoxia and fatiguing exercise^{33,42}. Taken together, this means that a condition of hypoxia, even a relatively normoxic oxygen supply-demand mismatch, will cause an increase in glycolytic flux and an increased rate of lactic acidosis resulting in a fall of muscle pH, probably leading to contractile fatigue⁸.

Unfortunately, examination of any standard biochemistry textbook⁴⁸ again turns up a problem. The section describing the enzyme phosphofructokinase will almost certainly describe the allosteric interaction between the catalytic activity of this enzyme and, among other factors, the pH. It has been axiomatic that the activity of this enzyme is drastically inhibited by hydrogen ions^{50,51,54}, which we know to accumulate and to result in a fall in muscle pH, when muscle is made hypoxic¹⁹. If there were such an allosteric inhibition of muscle PFK activity that muscle pH fell, lactate accumulation would not increase with exercise intensity or any increasingly severe oxygen supply-demand mismatch. Not only should this relationship have been obvious, it can be seen in many different types of studies. In conditions where the pH of the cell has been measured^{9,41} or could be estimated from the equations relating lactate concentration to pH^{1,15,45}, there is a continued production of lactate even as pH continues to fall¹⁴.

The integration of the intrinsic behavior of PFK and the overall function of glycolysis clearly supports the conclusion that PFK is *not* inhibited *in vivo* during conditions where pH falls¹¹, nor is it obvious that the fall in pH is even due to the accumulation of lactic acid^{10,17}. More importantly, the integration of glycolytic flux in general is clearly more complex than a simple ATP source during anaerobiosis^{6,7}. Overall, then, it is clear that the traditional view of glycolysis as an anaerobic path-

way is incorrect. However, the exact relationship between oxygen availability and glycolytic flux remains to be completely elucidated, in part due to the difficulties inherent in accurately measuring the intracellular PO_2 ^{5,21,39,47}.

Citric Acid Cycle

As above, if you open any biochemistry textbook⁴⁸, you will find a chapter entitled the citric acid cycle. Similarly, the overall structure of this chapter will be to take the student sequentially around this metabolic pathway, usually starting with the condensation reaction of oxaloacetate + acetyl-CoA to produce citrate. Again, if you examine each of these enzyme catalyzed steps carefully, you will see that none of these reactions require the participation of oxygen, although following the carbon skeleton around the citric acid cycle will uncover the production of nTP (ATP and GTP), reducing equivalents (NADH and FADH) and CO_2 . However, it is the accumulation of the reducing equivalents that provides the thermodynamic driving force for the normal function of the electron transport chain leading to the oxidative phosphorylation of ATP.

During hypoxia or any related oxygen supply-demand mismatch, normal ATP demand can exceed oxidative ATP production capacity⁴⁰. Since it was first postulated as an alternative mechanism by which skeletal muscle could generate ATP as a supplement to glycolysis during long periods of oxygen deprivation³⁸ a reverse-direction flux of the TCA cycle has been used to explain the regulation of metabolites during hypoxia in other tissues as well⁴⁹, associated with an accumulation of succinate. One of the distinctive features of exposure to acute hypoxia is such an accumulation of succinate. Succinate concentrations have been observed to increase after hypoxic episodes in yeast³⁷, liver⁵⁷, heart^{24,49} and muscle¹⁸. This increase in succinate concentration is associated with a general anaplerosis of the citric acid cycle which is linked to the rate of entry of glycogen derived pyruvate⁴³. Whether this is due to an energy-dependent mechanism or is simply the result of some sort of micro-compartmentation remains to be determined^{25,46}.

Once again, the integration of the behavior of the citric acid cycle in a normoxic condition versus its behavior during hypoxia is complex and begs the question of the exact purpose of the citric acid cycle. In normoxia, the traditional belief is that the citric acid cycle incorporates acetyl units and after the requisite number of unit turns of the cycle, the process of making CO_2 provides the reducing equivalents necessary for the normal functioning of the electron transport chain, and that any intrinsic production of nTP is of minor importance. It is intriguing that this might be fundamentally different during hypoxia. However, it is well known that the intrinsic affinity of mitochondria for oxygen is nearly infinite⁵⁵. If this is so, then the question of the functional meaning of hypoxia to mitochondria remains unanswered.

Oxidative Phosphorylation

The vast majority of the ATP produced by any mammalian tissue, here most importantly for muscle, is produced via mitochondrial oxidative phosphorylation^{21,22,32,36,58}. As described above, the intrinsic affinity of mitochondria for oxygen is quite high. Measured *in vitro*, the K_m of mitochondria for oxygen is on the order of 0.1 to 1.0 Torr⁵⁶. Thus, in the absence of any large impedance to the flow of oxygen down a concentration gradient from the vascular space (mostly O_2 bound to hemoglobin) to the outer mitochondrial membrane, the mean capillary vascular PO_2

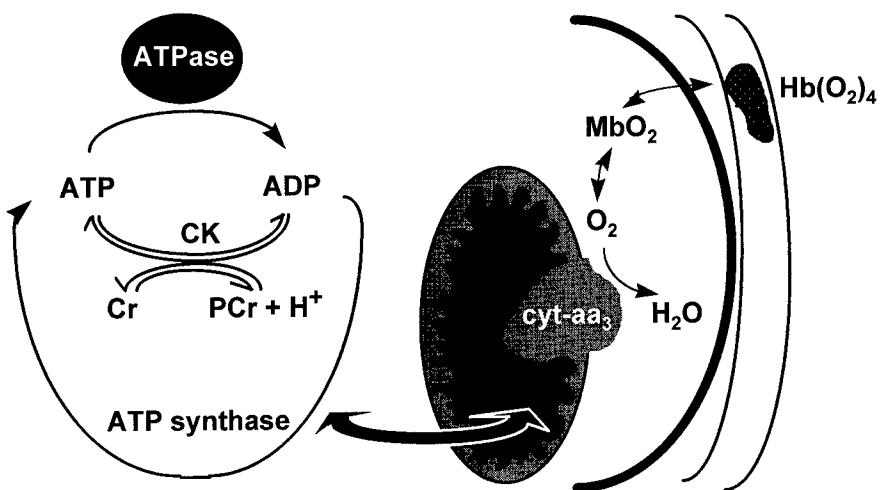


Figure 2 A model of skeletal muscle depicting the important sites where oxygen is involved in moving from the erythrocyte to the mitochondrial inner matrix. In most of these sites, it is currently impossible to accurately measure oxygen concentration without resort to at least some indirect assessments or assumptions.

would have to fall to near 1 Torr before there would be any attenuation of oxygen consumption due to frank oxygen unavailability.

However, the PO_2 of venous blood never falls that low. When you consider the normal limits of arterio-venous oxygen difference during exercise³⁵, it is difficult to imagine a mean capillary PO_2 that would be low enough to cause any attenuation in oxygen consumption solely from oxygen unavailability. Therefore, there must be at least one step between the oxygen bound to hemoglobin and the oxygen participating in the terminal oxidation step of the electron transport cycle that imposes a diffusion limitation. It is unlikely that this step is myoglobin^{31,52}. Therefore a reasonable supposition is that it is the endothelial space (the membranes between the vascular space and the inner sarcolemmal membrane). What remains to be done is to make accurate measurements of the oxygen content of each component of the system that is involved in the movement of oxygen from the erythrocyte to the outer mitochondrial membrane (Fig. 2).

Summary

Taken together, it is evident that the integration of metabolism and oxygen availability (Fig. 3) can be extraordinarily complex. In particular, the difficulties of making accurate measurements of oxygen content make understanding some of these systems impossible. From the earliest days of using the respiratory quotient to estimate metabolic balance² to modern spectroscopic methods of estimating molecular oxygenation^{23,30,34}, there is great need for accurate assessment of tissue oxygenation, both for clinical as well as basic science purposes. It is hoped that integrating the results of applying combinations of modern spectroscopies with modern biochemical techniques will allow us to better understand the fundamental role of oxygen to metabolic regulation.

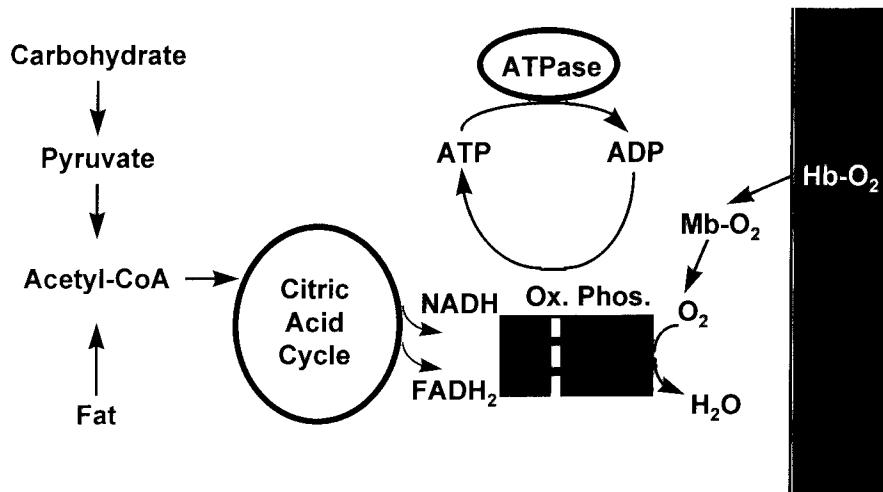


Figure 3 A model of the interaction between intermediary metabolism and oxygen movement from the vascular space to the mitochondria to provide a pathway for terminal oxidation of carbon to CO_2 with the concurrent production of the chemical energy of the terminal phosphate ester bond of ATP.

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CHAPTER 8

TRANSPORT OF OXYGEN IN MYOGLOBIN-CONTAINING MUSCLE

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Introduction

Oxygen is required for the aerobic synthesis of ATP, which provides energy to specific ATPases, those required to drive muscle contraction and the ionic pumps which maintain work output of heart and muscle. The rate of oxygen delivery to cytochrome oxidase at the inner mitochondrial membrane is a major determinant of the rate of work output of muscle and heart. In skeletal muscle and heart, oxygen carried by red blood cells must be released from combination with hemoglobin, diffuse across the red cell membrane, the surrounding layer of blood plasma, the capillary wall, the interstitial space, the sarcolemma and finally the sarcoplasm to reach the mitochondrial outer membrane.⁶ We will give an overview of the mechanisms by which oxygen is transported from the capillary blood to the mitochondria of myoglobin-containing tissues. In this context we will discuss new work (Sections IV, V, VI below) which describes the effects of intracellular myoglobin on the work output of cardiac cells, on maintenance of steady state levels of intracellular P_{O_2} , and on the maintenance of intracellular mitochondrial NADH.

I. Extracellular Oxygen Transport From the Capillary Red Blood Cell to the Muscle Cell Sarcolemma.

a) *Diffusive Flux.* Oxygen, reversibly bound to hemoglobin (Hb) in the red blood cell, is in equilibrium with free dissolved oxygen. Oxygen is released from HbO_2 when the plasma partial pressure of oxygen is lowered by oxygen uptake, and the partial pressure of CO_2 is increased, during tissue metabolism.

Oxygen, dissociated from erythrocyte hemoglobin, is delivered to the tissue cells by diffusion, the largest barriers being the capillary endothelial cells and the interstitial space between the capillary and the cell. The rate of diffusion is described by Ficke's law

$$-J = D^*A (c_0 - c_s)/x$$

where J is the magnitude of the flux, D is the effective diffusion coefficient of oxygen in this milieu, A is the surface area through which diffusion occurs, c_0 is the oxy-

gen pressure at the luminal face of the capillary wall, c_s is the oxygen pressure at the sarcolemma and x is the diffusion distance. With D , A and x constant, the diffusive flux of oxygen depends on the oxygen pressure difference ($c_0 - c_s$) from the capillary to the cell. This oxygen pressure difference, more than 20 torr in myoglobin containing tissues, provides a large force driving oxygen out from the capillary.

II. Intracellular Oxygen Transport From the Sarcolemma to the Mitochondria.

a) *Diffusive flux.* Oxygen, relatively insoluble in aqueous media, readily crosses the hydrophobic sarcolemma. Free dissolved oxygen is transported to the mitochondria during respiratory oxygen uptake by simple diffusion governed by the oxygen pressure difference from the inside face of the sarcolemma to the inner mitochondrial membrane. Figure 1A shows that in a heart cell, 20 μm across, in the absence of myoglobin, when oxygen transport is by diffusion alone, sarcoplasmic P_{O_2} would fall to low values at the center of the cell, and mitochondrial oxidative phosphorylation will become oxygen limited in this region. Conley² describes similar conclusions based on calculations of diffusive flux based on morphometry of horse and steer muscle operating at high work loads.

b) *The role of intracellular myoglobin in oxygen transport in heart.* Heart cells contain a substantial concentration of intracellular myoglobin, about 200 μM . Deoxygenated myoglobin in the sarcoplasm readily combines with oxygen in a near-equilibrium reaction. Since myoglobin is a monomer there are no allosteric modulations of myoglobin oxygenation.



As a consequence of oxygen binding by deoxymyoglobin at the inner surface of the sarcolemma, the partial pressure of free oxygen is diminished, the oxygen gradient between the capillary and the sarcoplasm is steepened, and the diffusive flux of oxygen into the cell is increased (Fig. 1). Oxymyoglobin will diffuse away from the sarcolemma into the sarcoplasm, driven by the gradient of myoglobin oxygenation from the sarcolemma to the innermost mitochondrion of heart and of myoglobin containing skeletal muscle (Fig. 2). Oxygen is released from oxymyoglobin at the outer mitochondrial membrane as a consequence of O_2 uptake at the inner mitochondrial membrane, thus creating a gradient of oxymyoglobin from the sarcolemma to the outer mitochondrial membrane and a gradient of deoxymyoglobin in the opposite direction from the outer mitochondrial membrane to the sarcolemma.

Oxygen diffuses through the sarcoplasm as free dissolved oxygen, a flux driven by the oxygen pressure gradient from the sarcolemma to the inner mitochondrial membrane. Additionally oxygen is carried pick-a-back as myoglobin-bound oxygen, generating a flux of myoglobin-bound oxygen from the sarcolemma to the outer mitochondrial membrane. The total flux of oxygen through a given area, in the presence of intracellular myoglobin during respiratory oxygen flux is described as:

$$-J = D_{\text{Mb}} C_{\text{Mb}} (Y_s - Y_m)/x + D (c_s - c_m)/x$$

where D_{Mb} = the diffusion coefficient of myoglobin, C_{Mb} = the concentration of myoglobin, c_s and c_m are the oxygen concentrations at the sarcolemma and at the mitochondria respectively, and Y_s and Y_m are the fractional saturation of myoglobin with oxygen at the extremes of the oxygenation gradient from the inner surface of

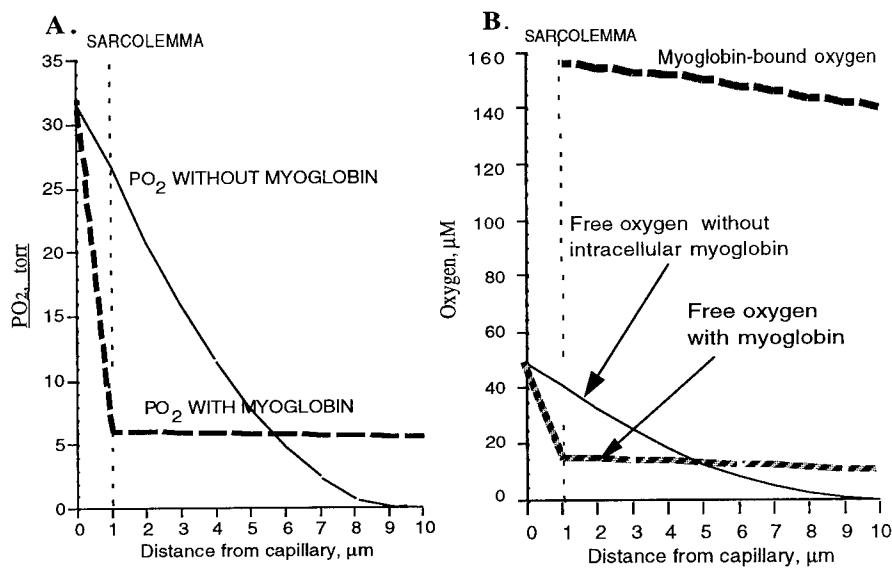


Figure 1 Schematic representation of oxygen pressure and oxygen concentration gradients from the outside of the capillary to the center of a cardiac cell.

A. Oxygen pressure gradients The mean fractional saturation of intracellular myoglobin is 70%, as measured in the blood perfused heart.⁷ The sarcoplasmic P_{O_2} without myoglobin was modeled assuming multiple sites of homogeneously distributed O_2 consumption. The diffusion equation with a homogeneous sink term is solved numerically and propagated in time until an equilibrium value of O_2 was achieved across the 10 μm distance halfway into the cell. (D. Eads personal communication). Diffusion of oxygen through the hydrophobic sarcolemmal membrane is expected to be rapid and is neglected for this analysis. The partial pressure of oxygen within the sarcoplasm, remains nearly constant in the presence of myoglobin, but in the absence of myoglobin, drops below the critical P_{O_2} for cytochrome oxidase (0.2 torr) towards the center of the cell. Mitochondria in this region would be oxygen-limited in the absence of myoglobin.

B. Oxygen concentration gradients from the capillary wall through the sarcolemma to the innermost mitochondrion at the center of a cardiac cell. Assumptions as in A. Note that in the presence of myoglobin the amount of oxygen carried by oxymyoglobin is far greater than that of oxygen free in solution. However the amount of oxygen stored as oxymyoglobin is only enough to supply the oxygen demand of the beating heart for several heart beats.

the sarcolemma to the outer mitochondrial membrane. Thus the total flux of oxygen is equal to the sum of the flux due to simple diffusion and that due to myoglobin-mediated oxygen transport (facilitated diffusion) (Fig. 2).^{6,15,17} The contribution of myoglobin-mediated oxygen flux was calculated to contribute about one-third of O_2 demand in skeletal muscle.² In isolated heart cells, about 30% of O_2 uptake is dependent on functional myoglobin.¹⁴

It has been shown recently by microspectrophotometry⁴ and by H^+ NMR⁷ that in the well oxygenated blood perfused beating heart, intracellular myoglobin is maintained in the partially deoxygenated state. Consequently deoxymyoglobin is present and available to bind incoming oxygen. In the presence of myoglobin in the partially deoxygenated form, the partial pressure of oxygen in equilibrium with myoglobin remains nearly constant, and well above the critical PO_2 required to maintain the maximum activity of cytochrome oxidase in the cell.^{2,4,7,15} In the absence of myo-

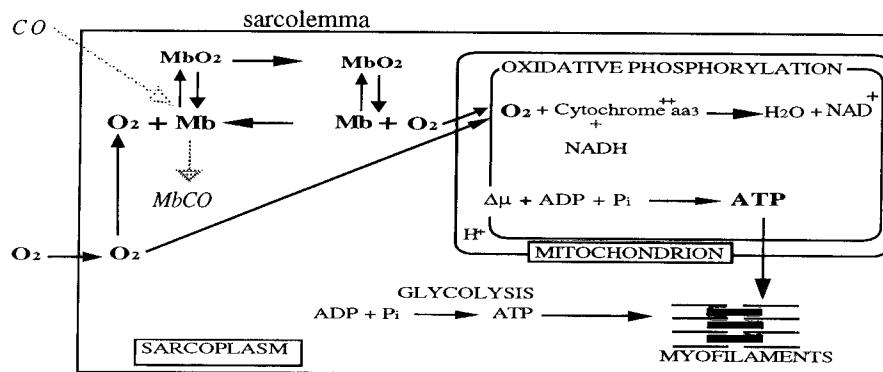


Figure 2 Simplified schematic representation of oxygen delivery, oxygen buffering and oxygen uptake reactions in a cardiac cell during contraction.

Equilibrium of oxygen with myoglobin prevents local depletion of oxygen pressure near the mitochondrial surface. Oxygen arrives at the outer mitochondrial membrane by two routes: diffusion of free oxygen and diffusion of myoglobin-bound oxygen (myoglobin-facilitated diffusion of oxygen) and oxygen then diffuses to cytochrome oxidase in the inner mitochondrial membrane. ATP is generated by mitochondrial oxidative phosphorylation and to a lesser extent by sarcoplasmic glycolysis. The ATP level is buffered by the near equilibrium reaction with creatine phosphokinase. The dotted arrows indicate the pathway of sequestration of myoglobin by tight competitive binding of CO. When myoglobin is sequestered in the CO form, facilitated diffusion vanishes. Cytochrome oxidase is not inhibited at the ratio of CO/O₂ used in our experiments.¹⁴

globin, mitochondria throughout the sarcoplasm will take up oxygen, and near the center of a heart cell, (7-10 mm from the sarcolemma), sarcoplasmic PO₂ may drop to values sufficiently low to limit the rate of oxygen uptake of the innermost mitochondria in heart (Fig. 1) and in skeletal muscle.² Figure 1B presents the intracellular oxygen levels expressed as concentration rather than partial pressure. This display shows that the amount of oxygen bound to myoglobin in cells of the beating heart is about 30-fold greater than the amount of oxygen free in solution. Thus this reserve provides a capacitance which permits the cell to draw briefly on intracellular oxygen reserves during changes in the oxygen demand, without large changes in the intracellular oxygen pressure. The amount of oxygen stored as oxymyoglobin is only enough to supply the oxygen demand of the beating heart for several beats, if the oxymyoglobin stores are not replenished from the capillary blood.

Myoglobin can not cross the mitochondrial membrane. Instead, arriving oxymyoglobin molecules, constantly replenish oxygen consumed by cytochrome oxidase and thereby prevent the local oxygen pressure from dropping to near zero. Consequently the oxygen pressure at the mitochondrial surface, albeit small, is maintained well above the critical PO₂ required to drive diffusing oxygen the short distance to the cytochrome oxidase in the inner mitochondrial membrane. Oxygen enters the mitochondrion by simple diffusion, and this diffusive flux is equal to the sum of oxygen released from oxymyoglobin and oxygen arriving by simple diffusion of dissolved oxygen through the sarcoplasm. (See Fig. 2). The total flux is sufficient to maintain the maximal activity of the cytochrome oxidase in the cell.^{2,4,15}

Thus myoglobin serves four important functions in intracellular oxygen transport:

- 1) By combining with oxygen just inside the sarcolemma, myoglobin reduces intracellular P_{O_2} and enhances the diffusion gradient for oxygen from the capillary wall and the sarcolemma.
- 2) Myoglobin provides an additional flux of myoglobin-bound oxygen from the sarcolemma to the mitochondria.
- 3) Myoglobin maintains a near constant value of sarcoplasmic P_{O_2} (provides O_2 capacitance), which defines the diffusion gradient from capillary to sarcoplasm.
- 4) Myoglobin maintains an oxygen pressure at the outer mitochondrial surface sufficient to drive the required flux of oxygen from the outer mitochondrial surface to cytochrome oxidase located in the inner mitochondrial membrane.

III. Oxygen Utilization at the Inner Mitochondrial Membrane.

a) *Mitochondrial NADH*: The level of mitochondrial NAD reduction to NADH reflects the balance between oxygen delivery and oxygen uptake at the mitochondria when substrate supply is constant (Fig. 3). Mitochondrial NADH levels increase progressively when ambient oxygen pressure is reduced, up to 100% of total mitochondrial NAD in the absence of oxygen.

b). *The rate of oxygen uptake and ATP synthesis*. Oxygen delivered to ferrous cytochrome oxidase at the inner mitochondrial membrane is required for oxidative phosphorylation, leading to the synthesis of ATP.

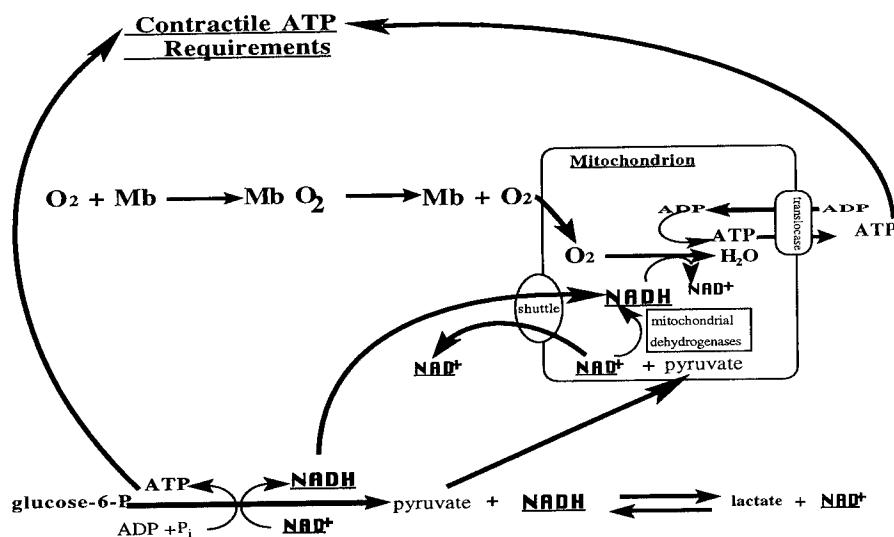
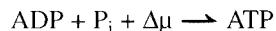


Figure 3 Schematic of sarcoplasmic reactions which supply oxygen, substrate and NADH to mitochondria for the synthesis of ATP.

In the mitochondria, oxygen, NADH and ADP are converted to H_2O , NAD^+ and ATP respectively. ATP is used for increased ATP utilization at the myofilaments, as well as for increased rates of ion pumping during enhanced contractile activity.

Mitochondrial NADH is replenished by substrate dehydrogenation, and is oxidized by the electron transport chain in the presence of oxygen. ATP synthesis utilizes the energy of the electrochemical membrane potential formed during electron transport along the mitochondrial chain. The mitochondrial NADH level is in near equilibrium with the mitochondrial electrochemical potential.

The net reactions of the electron transport chain during oxygen utilization are: oxidation of mitochondrial NADH by dissolved oxygen, maintenance of the electrochemical gradient across the mitochondrial inner membrane ($\Delta\mu$) and synthesis of ATP from ADP and $\Delta\mu$ by the mitochondrial ATP synthase. To maintain steady state levels of mitochondrial NADH and $\Delta\mu$, the rate of oxygen uptake (determined by the rate of ATP synthesis) must be balanced by the delivery of oxygen and substrate to the mitochondria.



Regeneration of NADH from NAD⁺ is linked to substrate dehydrogenation in both the sarcoplasm and the mitochondria; oxygen is delivered both by simple diffusion to the mitochondria and by release of oxygen from MbO₂ at the outer mitochondrial membrane; and ADP is delivered through the outer mitochondrial membrane by the specific ADP/ATP translocase. ATP in turn is utilized to drive tissue ATPases, including ion specific ion pump ATPases and actomyosin ATPase which are required to maintain the work output of heart and muscle. The ATP level is maintained nearly constant by near equilibrium reaction with phosphocreatine, catalyzed by creatine phosphokinase. Thus in the working muscle, depletion of energy reserves is signaled by a drop in phosphocreatine. A portion of the energy requirement of working tissue may be met by ATP generation from glycolysis, but the yield of ATP produced by oxidative phosphorylation, 34 ATP formed per mol glucose is much greater than that, 2 ATP formed per mol glucose, produced during glycolysis (Fig. 3).

IV. Stimulated Energy Output: The Role of Myoglobin

a) *Skeletal Muscle*: Heart and skeletal muscle must rapidly adapt their oxygen uptake to meet the increased energy demand from rest to exercise. It has been suggested that myoglobin is present in those skeletal muscle fibers whose maximal rate of work output can not be met by the rate of simple oxygen diffusion.² Myoglobin-dependent oxygen diffusion requires that oxymyoglobin molecules are free to move in the sarcoplasm. The macroscopic diffusion coefficient of intracellular myoglobin in skeletal muscle has been shown to be much slower than expected from measurements in dense protein solutions.⁸ However the microscopic cellular diffusion coefficient, not measured by this technique, may be the effective parameter.⁸ When intracellular myoglobin is specifically inactivated, without inhibiting other heme proteins of the mitochondrial cytochrome chain, work output and oxygen uptake of the *in situ* gastrocnemius muscle are significantly and reversibly diminished but not abolished.¹ An effect of intracellular myoglobin on the work output of beating mammalian heart cells has not previously been described.

b) *Isolated heart cells*: The work output of the heart is measured by the rate-pressure product (heart rate \times developed pressure). We are able to monitor the amplitude and rate of contraction of unloaded, isolated heart cells during pacing as an index of cellular work output. A video camera and a video edge detector were used to measure the amplitude and frequency of contractions. We were able to demonstrate for the first time in mammalian heart, that the amplitude of contraction of isolated heart cells electrically stimulated to contract at 4 Hz significantly and

reversibly decreases when intracellular myoglobin is inhibited by CO.¹² When myoglobin function is specifically abolished with CO, the amplitude of contraction is diminished to about 60% of control, and contraction amplitude becomes erratic. On the other hand, when myoglobin function is intact the amplitude of contraction of isolated cells is not diminished when glycolysis is inhibited by the addition of deoxyglucose, and the cells are made totally dependent on oxidative phosphorylation for their ATP supply. When myoglobin is inactivated by CO in the presence of deoxyglucose, the diminished amplitude of cell contractions is exacerbated over that observed with glycolysis intact. In this case the amplitude of contraction is diminished to 25% of that observed in the presence of deoxyglucose in the presence of functional myoglobin. When sodium nitrite is used to inactivate intracellular myoglobin by conversion of myoglobin to the high spin ferric form, a similar reversible decrease in the amplitude of contraction is observed, and in addition, cell contractions no longer follow the rate of stimulation at 4 Hz. These cells contract at 2 Hz when electrically stimulated at a rate of 4 Hz.

c) *Isolated mitochondria*: In the absence of myoglobin, oxygen consumption and ATP synthesis by the tightly-coupled, isolated mitochondria of rat or pigeon heart becomes oxygen limited when P_{O_2} drops below the critical P_{O_2} for cytochrome oxidase. Myoglobin, added to the mitochondrial suspension at low P_{O_2} , restores the rate of oxygen uptake and ATP formation to 100% of maximum. The effect of added myoglobin reaches a maximum near 50 μM . With a variety of oxygen transporting proteins it has been demonstrated¹⁶ that the unique property required to enhance oxidative phosphorylation is the ability to maintain the P_{O_2} at the outer mitochondrial membrane above a critical limiting value, $P_{1/2} = 0.05 - 0.1$ torr. Intracellular myoglobin performs this function in intact tissue.^{4,7}

V. Changes in Steady State Intracellular Parameters During Increased Energy Output

Myoglobin oxygenation: Since intracellular myoglobin is in equilibrium with dissolved oxygen, the steady state level of myoglobin oxygenation is a direct intracellular measure of the sarcoplasmic P_{O_2} . It is possible to measure the extent of intracellular myoglobin oxygenation by H^+ NMR.⁹ We have used this technique to measure intracellular myoglobin oxygenation of the blood perfused, isovolumic beating heart during periods of increased exercise.⁷ We found that the oxygenation of intracellular myoglobin is sensitive to changes in ambient oxygen pressure below 21% oxygen. In contrast, myoglobin oxygenation, and therefore the intracellular oxygen partial pressure, remained remarkably constant when heart rate was increased up to 10-fold. Thus in the presence of functional myoglobin, the balance between oxygen supply to tissue cells and oxygen uptake is maintained when the work output and oxygen uptake is increased 10-fold.

Mitochondrial NADH: Mitochondrial NADH was measured in perfused isolated heart cells by microfluorescence.¹⁰ During 4 Hz pacing, mitochondrial NAD is less reduced (more oxidized) and the oxygen uptake is stimulated 7-fold.¹¹ Mitochondrial NADH does not decrease during increased pacing when intracellular myoglobin is inactivated by nitrite¹⁰ or CO.¹³ Thus in the absence of functional myoglobin, mitochondrial NADH is increased during 4 Hz stimulation compared to mitochondrial NADH under the same conditions in the presence of myoglobin. This response is similar to that observed when paced heart cells are superfused with

hypoxic medium equilibrated with 20% oxygen.¹⁰ Previously we showed that inactivation of intracellular myoglobin of isolated heart cells reduces the respiratory oxygen uptake by about 30%.¹⁴ The simplest explanation of the data is that in the absence of functional myoglobin, intracellular mitochondria become oxygen limited during pacing, and the rate of oxygen uptake and of ATP synthesis is decreased.

Taken together with the decrease in contraction amplitude, these findings suggest that rapidly paced cells without functional myoglobin, although well oxygenated, demonstrate a functional impairment in oxygen delivery which limits the rate of ATP synthesis. As a consequence, the ATP requirement for maximal amplitude of contraction is not met. The simplest explanation of these findings is that functional myoglobin enhances the flux of oxygen to cardiac mitochondria during exercise, and that, in the absence of functional myoglobin, the rate of oxidative phosphorylation is limited by insufficient flux of oxygen to the mitochondria even when the ambient oxygen concentration is high.

High energy phosphate levels: We find that the high energy phosphate levels of isolated heart cells are unchanged during 4 Hz stimulation when intracellular myoglobin is functional. Phosphocreatine levels of isolated heart cells decrease when myoglobin is inactivated with sodium nitrite.^{3,5} Furthermore, we now find that the phosphocreatine/ATP ratio is significantly decreased ($p = .0043$, $n = 7$ different experiments) during 4 Hz stimulation when myoglobin is inactivated with sodium nitrite.

Thus when work output is challenged by pacing cells with inactivated myoglobin, steady state phosphocreatine, and mitochondrial NADH levels are the first indicators of change in energy balance.

VI. Hypoxia

When oxygen equilibration of the blood or saline perfusing the heart is experimentally curtailed, there is a decrease in the contractile activity of the heart culminating in cessation, mitochondrial NADH levels increase, and high energy phosphate levels decrease. It is suggestive that these symptoms are also observed in heart or heart cells stimulated to contract in the absence of functional myoglobin. However it should be noted that when myoglobin is inactivated, even when glycolytic ATP production is inhibited, diminished heart cell contraction is maintained since the diffusive flux of oxygen is unimpaired.

Summary:

(1) Myoglobin, always partially deoxygenated in the beating heart, maintains a constant steady state level of intracellular oxygen pressure in the blood perfused beating heart. (2) In the absence of functional myoglobin, the amplitude of contraction of isolated heart cells during 4 Hz stimulation is significantly decreased. (3) In the absence of functional myoglobin, mitochondrial NADH is significantly increased (less oxidized) during pacing compared to control cells with intact functional myoglobin. (4) High energy phosphate levels of isolated cells are significantly decreased during 4 Hz stimulation when myoglobin is inactivated. In the absence of functional myoglobin, heart cells are functionally oxygen limited even when they are provided with abundant ambient oxygen. Functional intracellular myoglobin is necessary to maintain maximal contraction in the beating heart.

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CHAPTER 9

THE REGULATORY ROLE OF MYOGLOBIN IN MYOCARDIUM

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Introduction

In the general view, Mb is either an oxygen storage protein, ready to compensate any cellular oxygen deficit, or is a facilitator of oxygen diffusion.^{1,2} Some recent studies, however, have proposed that Mb may indeed play a direct role in regulating oxygen consumption. Under oxygenation conditions that far exceed mitochondrial demand, nitrite or carbon monoxide inhibition of Mb function will produce a decline in oxygen consumption (MVO_2) and phosphocreatine (PCr) in the myocytes, even though cytochrome aa₃ reduction state remains unperturbed.³⁻⁵ That observation suggests that Mb inhibition may modulate directly oxidative phosphorylation.

Much of the supporting data for a direct Mb role originate primarily from myocyte experiments, which utilize nitrite or CO inhibitors of Mb. However nitrite studies of perfused myocardium results have yielded contrasting results.^{4,6,7} The disparity between myocyte and perfused heart studies may arise in part from the functional differences in the model systems but also from the uncertain extent of nitrite or carbon monoxide inhibition of Mb, since assessing Mb oxidation or CO binding *in vivo* poses a technical challenge. Optical techniques cannot distinguish easily the signals of MbO₂, MbCO, and oxidized Mb saturation in a beating heart.^{8,9}

In contrast the ¹H NMR CH₃ Val E11 signal can reflect both the intracellular pO₂ as well as the pCO. At -2.76 ppm the signal of MbO₂ reflects the oxygenated state and decreases its intensity upon deoxygenation.¹⁰ With increasing pCO, the MbO₂ signal declines as the corresponding MbCO signal emerges at -2.26 ppm, (Fig. 1). Moreover, the same spectral region exhibits a signal at -3.9 ppm, which reflects the transition between the Fe (II) and Fe (III) states of Mb.⁷ The ¹H NMR signals then provide a unique assessment of the extent of Mb inhibition state and form a critical basis for exploring the role of Mb in the cell.

Along with the deoxy Mb proximal histidyl N₈H peak, the Mb signals can provide a unique glimpse into the function of Mb in regulating oxygen consumption demand under different physiological conditions.¹⁰ Recent studies have demonstrated that the signals are detectable directly from *in situ* myocardium.

CO Binding to Myoglobin *in vivo*

Although CO binding to Mb *in vitro* is well characterized, the ligand binding *in vivo* is not. The myocardial response to increasing pCO is reflected in the ¹H and

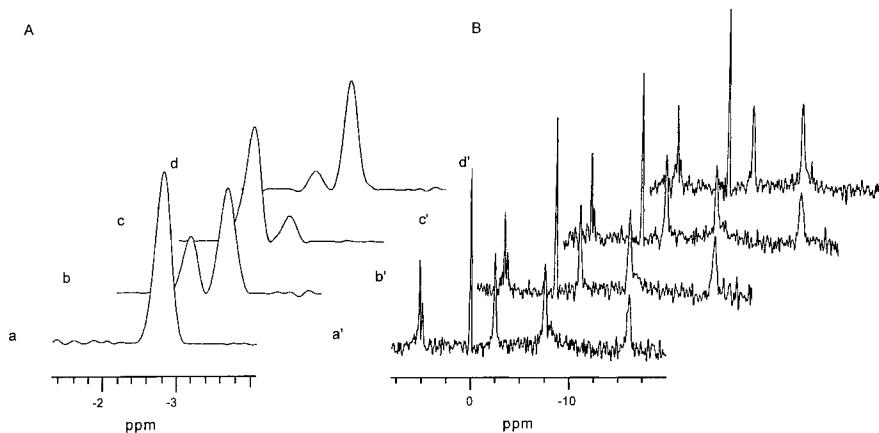


Figure 1 ^1H and ^{31}P NMR spectra of perfused myocardium perfused under different partial pressures of carbon monoxide (CO): **A.** The ^1H spectra are shown from a-d a) pCO 0 torr: CO free, oxygen saturated buffer flowing at 18 ml/min. The γCH_3 Val E11 signal of MbO_2 appearing at -2.76 ppm reflects the fully oxygenated state. b) pCO 14.2 torr. The γCH_3 Val E11 signal of MbO_2 decreases, but the signal corresponding to the γCH_3 Val E11 of MbCO emerges at -2.26 ppm. c) pCO 65.6 torr. The MbO_2 signal is significantly reduced, while the signal of MbCO at -2.26 ppm becomes prominent. d) Reperfusion with CO free, oxygen saturated buffer. The MbCO and MbO_2 signals are in dynamic equilibrium. Signal intensity loss in one peak corresponds to the signal intensity gain in the other. **B.** The corresponding ^{31}P spectra are shown from a'-d'

^{31}P spectra, (Fig. 1A and 1B). During the control period the ^1H NMR spectra from well oxygenated myocardium exhibit a distinct γCH_3 Val E11 signal of MbO_2 at -2.76 ppm (Fig. panel 1A).^{7,11,12} As the pCO of the perfusate increases, the MbO_2 signal decreases, while the corresponding MbCO peak at -2.26 ppm increases, (Fig. 1A[b-c]). Upon reperfusion with 95% O_2 /5% CO_2 saturated buffer, the MbO_2 signal recovers while the MbCO signal decreases, (Fig. 1A[d]). In contrast no change is detectable in the corresponding ^{31}P NMR spectra, (Fig. 1B[a'-d']).

Throughout a range of pCO, a dynamic equilibrium exists between MbO_2 and MbCO with no significant intervening Mb species. A plot of the fractional MbCO/MbO_2 vs pCO/pO₂ yields a linear relationship and a partition coefficient, $P=[\text{MbCO}]\text{pO}_2/[\text{MbO}_2]\text{pCO}$, of 36, which is consistent with the observation from *in vitro* studies.^{5,13,14} Any vasculature based discrimination of CO/O₂ delivery or transport to the cell appears insignificant. The *in vivo* MbCO off rate kinetics is shown in Figure 2A. Upon reperfusion with oxygenated buffer, the 84.9% saturated MbCO declines to 50 % of its original intensity within 10 minutes, indicating an apparent first order k_{off} of $1.2 \times 10^{-3} \text{ sec}^{-1}$ at 37°C. The *in vivo* k_{off} value is somewhat lower than the *in vitro* value of $1.7-4 \times 10^{-2}$ at 22°C.

The physiological/metabolic impact of CO in the cell is somewhat unexpected. Up to 80% of MbCO saturation, the MVO₂ remains constant, while RPP declines 10% well below the [pCO]₅₀ of Mb. The MVO₂ and RPP response to varying pCO is shown in Figure 2B. Despite an adequate oxygen supply and a constant MVO₂, the myocardium still stimulates lactate formation rate by a factor of 2 when MbCO saturation reaches 75%, well below the reported CO/O₂ ratio required to inhibit cytochrome oxidase.^{8,14,15} No shift in MVO₂ appears until the MbCO saturation approaches 90%. PCr, ATP, pH, and Pi levels remain constant throughout. Quite

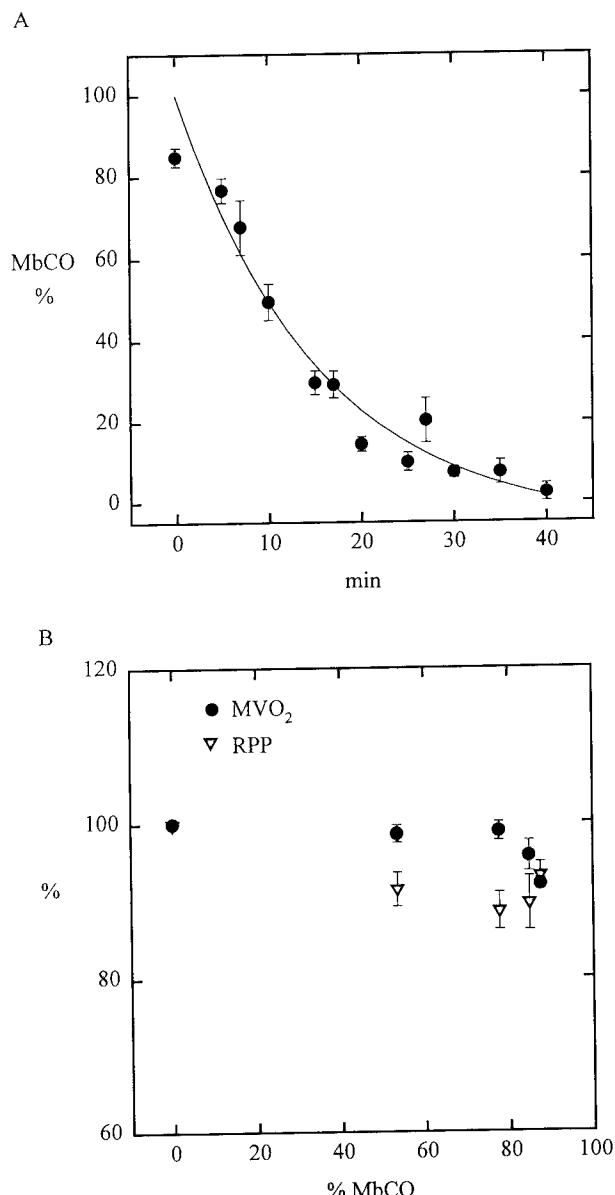


Figure 2 Kinetics of MbCO release in the cell and the RPP as well as MVO₂ relationship as a function of MbCO saturation: **A.** Time course of the MbCO dissociation in the myocardium upon reperfusion with oxygen-saturated buffer. The apparent first order off rate time constant is 1.16×10^{-3} . **B.** Graph of MVO₂ (●) and RPP (▽) vs percent Mb saturation. MVO₂ shows no change until MbCO saturation exceeds 80%, while RPP has already exhibited a significant decline.

clearly a highly energized cellular state, as reflected by the ³¹P spectra, does not produce an initial decline in contractile function and an increase in lactate formation.

CO Effect on Regulatory Pathways

The CO induced RPP response appears in two phases: The initial drop shows no dose dependent response to MbCO saturation. At the Mb [pCO]₅₀, RPP has already decreased to a steady state level and does not decline further. Even though oxidative phosphorylation, as reflected in MVO₂ and PCr level, indicates that the bioenergetic status is still normal, the developed pressure has fallen. Such an observation of a direct CO effect points to a potential interaction with the heme protein, guanylyl cyclase. The research literature has substantiated that NO binding to guanylyl cyclase will trigger a reduction in myocardial contractile function. Whether CO will induce the same effect is presently moot. Some recent studies have suggested that CO can affect guanylyl cyclase, while others have contested the conclusion.¹⁶⁻¹⁹ Our observation of an MbCO independent RPP decline in the initial phase of CO infusion is consistent with a CO interaction with guanylyl cyclase.

In the second phase, the CO induced effect appears dependent upon MbCO saturation. Above 50% MbCO saturation, MVO₂, PCr, and ATP remain unaltered, but lactate formation has risen dramatically. RPP maintains its depressed, but steady state level. The lactate formation rate increases as a function of MbCO saturation in a dose dependent manner and implicates the presence of anaerobic ATP production. An enhanced glycolytic ATP production to meet an energy deficit in face of a normal oxidative phosphorylation rate would imply that the P/O ratio has shifted. Under all experimental conditions, the cellular pCO is insufficient to arrest the cytochrome oxidase activity. This view of Mb corresponds to its postulated role in directly regulating respiration.⁵

The MbCO experimental observations show a striking similarity to the myocardial response profile as oxygen levels decrease. Declining RPP and lactate levels mark the initial response to oxygen limitation, followed by alterations in MVO₂ and PCr.^{11,12} With CO perfusion, the myocardium still follows the same sequence, first decreasing its contractile function and increasing its lactate formation rate in responding to CO presence.

Nitrite Inhibition of Mb

Many experiments supporting a direct Mb role in respiration utilize myocytes and nitrite oxidation.³⁻⁵ Perfused hearts subjected to nitrite have not responded exactly as myocytes have responded. In fact nitrite infused myocardium reveals a complex interaction.¹² Even with 30 mM infused nitrite, a concentration that far exceeds the *in vitro* stoichiometry to oxidize Mb from Fe (II) to Fe (III), as reflected in the ¹H NMR signal at -3.9 ppm, MVO₂ shows no significant alteration, while RPP and PCr level decrease as lactate formation rate increases (Fig. 3, 4). Since MVO₂ is constant, the subsequent PCr loss in the face of a stimulated glycolytic ATP production or altered redox state suggests strongly that Mb may indeed mediate energy coupling in respiration.⁷

The myocardial experiments with nitrite, however, have not distinguished between a direct nitrite vs a Mb mediated mechanism. Nitrite itself can perturb the cellular metabolism, excluding a Mb participation. With carbon monoxide inhibition, the role of nitrite and Mb becomes clearer, since CO binds more tightly to Mb than O₂ but does not oxidize the heme Fe (II) to Fe (III). Even at 76.8% MbCO saturation, neither PCr nor MVO₂ is significantly impaired. Although these obser-

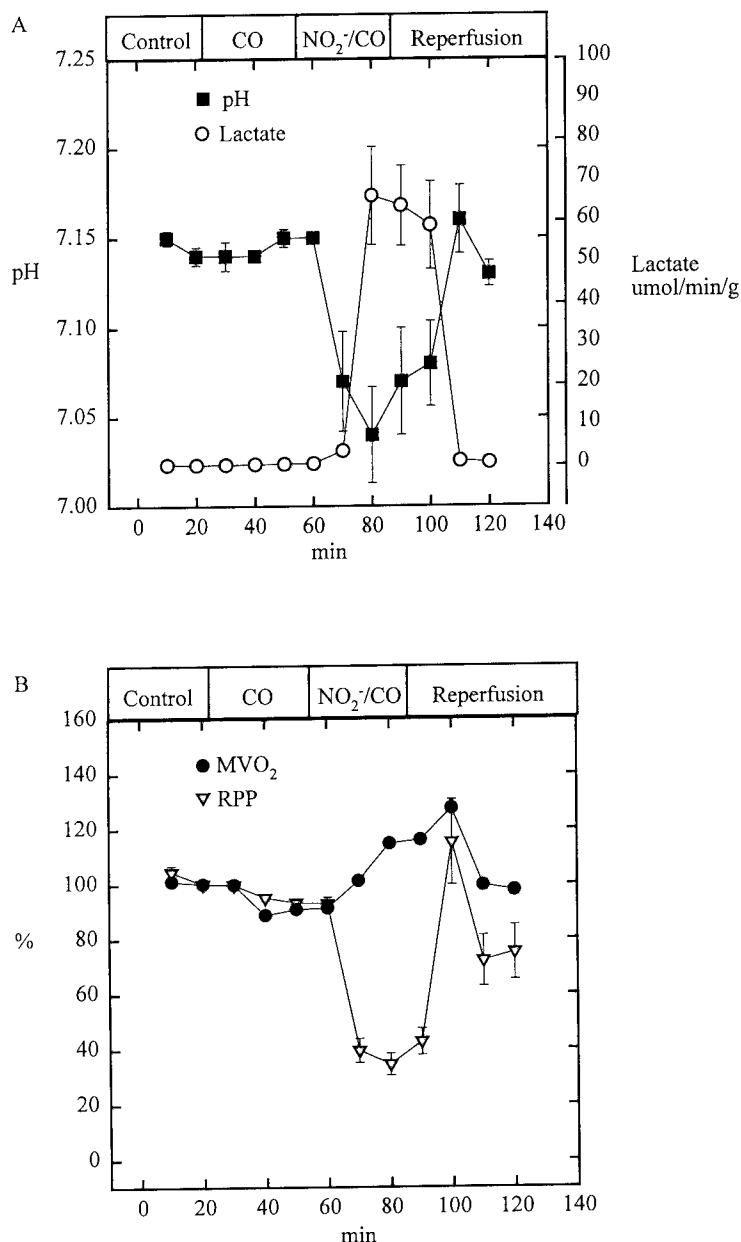


Figure 3 ¹H and ³¹P NMR spectra of perfused myocardium infused with varying concentrations of nitrite: **A**, The ³¹P spectra are shown from a-d. a) control b) 10 mM nitrite c) 30 mM nitrite d) reperfusion. **B**, The corresponding ¹H spectra are shown from a'-d'. a') Myocardium perfused with nitrite free, oxygen saturated buffer flowing at 16 ml/min. The γ CH₃ Val E11 signal of MbO₂ appears at -2.8 ppm and reflects full oxygen saturation. b') With 10 mM nitrite. A signal corresponding to metMb emerges at -3.9 ppm. c') With 30 mM nitrite. The -3.9 ppm signal of metMb becomes prominent. The signal intensity increase in the metMb signal is balanced by the decrease in the MbO₂ signal. d') Upon reperfusion with nitrite free, oxygen saturated buffer.

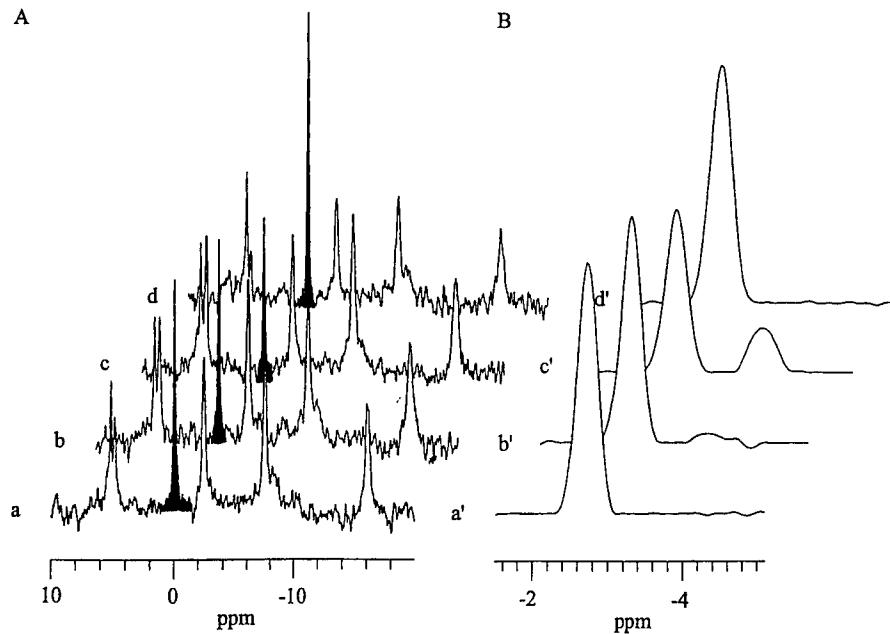


Figure 4 Graphs of the nitrite effect on the myocardial metabolic and physiologic responses when Mb is 86% inhibited by CO and cannot convert to metMb: **A.** CO does not perturb pH, but does elevate lactate formation rate by a factor of 2. With 50 mM sodium nitrite infusion, pH drops from 7.15 to 7.06, while lactate formation rate rises dramatically. Upon reperfusion with CO and nitrite free, oxygenated buffer, pH recovers to the control level, but lactate formation rate does not. Lactate (O) and pH () **B.** The corresponding \bar{MVO}_2 (●) and RPP () responses indicate that nitrite itself reduces the RPP level and gradually boosts the \bar{MVO}_2

vations are not completely consonant with myocyte results, which note a decline in both \bar{MVO}_2 and PCr above a 40% MbCO saturation threshold, they nevertheless indicate a potential uncoupling in oxidative phosphorylation, which may be dependent upon a Mb participation.^{3,4,7}

However, the magnitude of any Mb mediated interaction on respiration appears smaller than the direct nitrite effect, (Fig. 4). Upon infusion with 50 mM nitrite, the MbCO level remains constant, but RPP drops precipitously by about 50%, (Fig 4). No metMb signal is detected at -3.9 ppm. Meanwhile \bar{MVO}_2 increases slightly, whereas the lactate formation rate jumps dramatically up by a factor of 7. The cellular/metabolic response is quite similar to the one observed in nitrite perfused myocardium without CO, except that nitrite would have oxidized 33% of the Mb to metMb.⁷ The CO/NO₂ experimental results lead to the conclusion that nitrite itself can also mediate energy coupling and can trigger a lactate formation as well as oxygen consumption enhancement.

Future Perspective

The ¹H NMR Val E11 signal of Mb in myocardium can reflect its O₂, CO, and oxidized states and has led to insight into the potential role of Mb in directly regulating metabolism. Preliminary studies have already revealed that Mb can indeed

play a substantial role in mediating oxygen consumption. Vascular control does not appear sufficient to maintain the intracellular O₂ level constant. Moreover, the perfused heart experiment has established a definitive basis for further investigation of the Mb role in the intact animal. Both the Mb and Hb signals are detectable from the *in situ* rat heart. The signals are sufficiently well resolved at 7T to distinguish one component from the other. These results present a promising tool for future investigation into the control of oxygen consumption as well as the role of Mb *in vivo*.

Summary

The ¹H NMR myoglobin Val E11 signal provides a unique opportunity to observe Mb binding kinetics in the cellular environment, to detect carbon monoxide inhibition *in vivo*, and to assess the functional role of Mb in regulating oxygen transport as well as respiration. Upon carbon monoxide infusion in perfused myocardium, the MbO₂, Val E11 signal at -2.76 ppm gradually disappears, and a new signal, corresponding to MbCO, emerges at -2.26 ppm. These signals yield the intracellular partial pressure of both O₂ and CO. At 80% MbCO saturation, the contractile function has decreased, whereas the lactate formation rate has increased. Yet neither the PCr concentration nor the oxygen consumption rate is significantly perturbed. Above 80% MbCO saturation, the oxygen consumption rate starts to decline. Infusing nitrite does not produce any significant alteration in the MbCO signal intensity, but still alters both the MVO₂ and RPP. Nitrite appears then to affect myocardial metabolism and contraction by both a direct as well as an indirect Mb inhibition route. The CO/O₂ partition coefficient of 36 in myocardium is in good agreement with the *in vitro* value and implies no selective transport. The experimental observations support the hypothesis that Mb may have regulatory roles that are different from oxygen storage or facilitated diffusion. The perfused heart experiments have established a solid basis to utilize the Mb ¹H NMR signals to explore the role of Mb in regulating oxygen consumption in myocardium *in situ*.

Acknowledgments

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CHAPTER 10

NEAR INFRA RED

SPECTROSCOPY AND THE

CONCEPT OF A CRITICAL PO₂

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First of all I want to thank the organizing committee for inviting me and for allowing me so broad a title without putting boundaries on the subtopics I want to select. And select I will, partly because of time limitations, partly because this week our interest is focussed on Hypoxia—but mostly because I do best when talking about my primary interest: the reactions of cells with oxygen *in vivo*. This involves:

- * the enzyme that is the final, intracellular sink for oxygen;
- * hemoglobin in the microcirculation, the source of that oxygen;
- * the vascular system furnishing oxygen in the right amount;
- * the technique of non-invasive optical monitoring.

And since no organ is more dependent on a sufficient oxygen supply than the brain, that is the organ of choice for today's discussion. So there you have it: a large topic cut down to four items in a 25 minute sound bite.

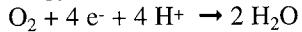
In this presentation we will attempt to elucidate some principles of non-invasive monitoring of cerebral oxygenation with emphasis on the enzyme that catalyzes the use of oxygen by the neurons. Cytochrome *c* oxidase (better known as cytochrome aa₃, abbreviated cyt aa₃) was described by David Keilin some seventy years ago as one of three components of a system of cell pigments that react with oxygen.¹⁰ Later findings showed that they are part of a series of single electron redox components that are instrumental in transferring reducing equivalents derived from the substrates (e.g. glucose) to oxygen. In the process the system harvests free energy that is made available, via oxidative phosphorylation, to endergonic cellular reactions in the form of high energy phosphate bonds. Thus the reactions of cyt aa₃ and especially its reduction/oxidation steady state (redox state) are of prime importance to understanding tissue hypoxia.

The enzyme possesses redox-state dependent absorption bands in the Near Infra Red region of the spectrum. This property has been turned to advantage since the NIR region allows optical observations of rather large organs such as the human head.⁵ A brief outline of the salient technical points of NIR Spectroscopy will be given further below after setting the stage with a bare outline of the enzyme's reactions. So please bear with me as I try to recapture the excitement of your first Biochemistry course.

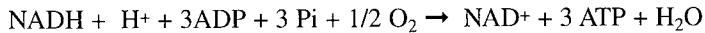
The respiratory chain is composed of a number of redox components that transfer reducing equivalents (in the form of hydrogen atoms and electrons) down a redox

potential gradient from -0.320 Volt to about +0.820 Volt. Figure 1 illustrates this route from the co-enzyme nicotine amide adenine dinucleotide (NADH) or from flavoproteins, such as succinic dehydrogenase, to molecular oxygen. The chain is primarily located in or on the inner membranes of the mitochondria and is composed of electron carriers. They can receive single electrons from a chain component with a more negative redox voltage and pass them on to an adjacent one that is more positive. The simplified diagram shown in the figure emphasizes the redox steps between the major, measurable components. The cyt aa₃ enzyme complex contains two iron redox components (hemes a and a₃) and two copper atoms, one associated with each heme. In the final reduction of oxygen to water by the cytochrome c oxidase complex, four electrons and four hydrogen ions are combined with one O₂ molecule, bound to the a₃-locus of the enzyme, to form two molecules of water. The enzyme, studied either in suspensions of mitochondria or with more purified preparations, possesses a very strong affinity for oxygen: a PO₂ of less than a millimeter keeps the hemes almost completely oxidized under active metabolic conditions.³

A large amount of free energy is available in the overall reaction



which resembles the explosive combination of oxygen with hydrogen gas. However, in the biological process the hydrogen, supplied by the substrates, is first dissociated into hydrogen ions and electrons. This reaction is often informally written as



emphasizing the production of three high energy phosphate bonds (~P) per oxygen atom used. It should be emphasized, however, that the transfer of single electrons to oxygen would create highly destructive oxygen radicals and that the presence of four redox centers, two heme irons and two copper atoms, each able to bind one electron, leads to the suggested mechanism that the four electrons are released at once during the reaction.⁷

At the three sites indicated in Figure 1, free energy is captured by the formation of a hydrogen ion gradient between the inside and outside of the mitochondrion. The gradient is then responsible for driving the reaction of adenosine di-phosphate (ADP) plus inorganic phosphate (P_i) and H⁺ to ATP. The required free energy is provided as the H⁺ ions slip back down their transmembrane gradient to the inner mitochondrial space.

This brief biochemistry refresher serves to illustrate the remarkable difference in the free energy driving high energy phosphate bond production at sites I & II and at

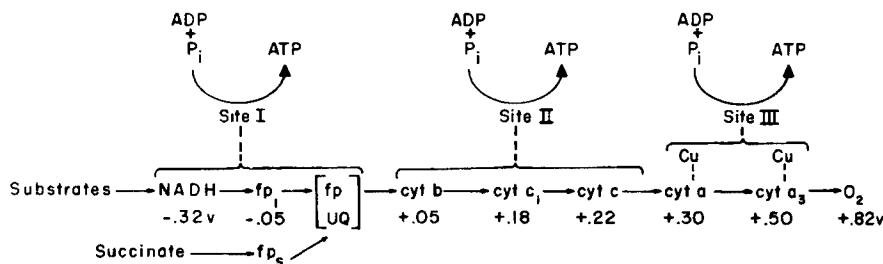


Figure 1 Simplified diagram of the respiratory chain emphasizing the energy conservation sites. The redox voltages given are the midpoint potentials.

site III, the terminal one involving cyt aa₃. There is twice as much energy available in the latter than in the first two, i.e. enough to produce one extra high energy bond. Mother Nature, not being capricious or occult, nor wasteful in her basic biological persona, demands we explain from current knowledge or by further experimentation, before we write off such a glaring difference to happenstance or hide behind the interdiction against teleological thought.

It is our working hypothesis that this extra energy, wasted in the *in vitro* determinations, is captured usefully *in vivo* in a manner closely allied to cellular function particularly ion transport.

Our insistence that differences between *in vivo* and *in vitro* results may be relevant, derives from a number of observations on intact tissues. Since cellular integrity is a prerequisite for understanding the integration of *in vitro* data into a model of cellular function, our experimentation depends primarily on methods that avoid trauma at the cellular level.

Non-invasive Optical Monitoring

Both the discovery by McMunn in 1884 of intracellular "haematin pigments" and the more detailed description of the cytochromes by Keilin in 1925 were based on visual observations of light absorption patterns using hand-held spectrometers. From then on the methodology lending itself best to monitoring the reactions of the system has proved to be the optical assessment of the redox state of key components of the chain. These components show characteristic differences in absorption bands depending whether they are oxidized or reduced. The optical approach was simplified upon the introduction by Chance in 1951 of rapid and sensitive electronic spectrophotometry of turbid samples.¹ Quantitative measurements were made routinely possible on optically non-ideal systems such as suspensions of red blood cells or of isolated mitochondria⁴ and excised muscles.² Early visual observations had restricted the wavelength range to the Visible part (VIS) of the spectrum. The range was soon extended to the near Ultra Violet (UV) for the monitoring of NADH with Chance's differential spectrophotometers. With the wavelength scanning technique the difference in absorption between two samples is recorded; the 'reference' usually in a stable, well oxygenated state, the experimental sample in a variety of conditions as called for in the protocol. Figure 2 shows the difference spectra of an anoxically reduced excised muscle vs its oxygenated partner used as a reference; the so-called reduced minus oxidized difference spectrum. The spectrum of cyt aa₃ shows two strong absorption bands; the one at 605 nm, i.e. in the orange part of the spectrum, is contributed for 80% or more by heme *a*, the one in the violet region at 445 nm is ascribed about equally to hemes *a* and *a*₃. Long after Keilin's rediscovery of the cytochromes by visual means, it was realized by Wharton and Tzagoloff¹⁷ that the enzyme also possesses a redox-sensitive absorption band in the NIR (Fig. 3). They ascribed it correctly to the copper atoms of the enzyme.

In vivo monitoring of respiratory chain components in the presence of blood is handicapped by the strong absorption bands of oxygenated and de-oxygenated hemoglobin (HbO₂ and Hb), especially in the near UV. In the visible region the exception is the 605 nm band of cyt aa₃ which can be monitored *in vivo* using a multi-wavelength method to compensate for both an increase in blood and in its degree of oxygenation. A three wavelength approach (or "triple lambda" method) can be used as long as the wavelengths are chosen quite closely together so as to

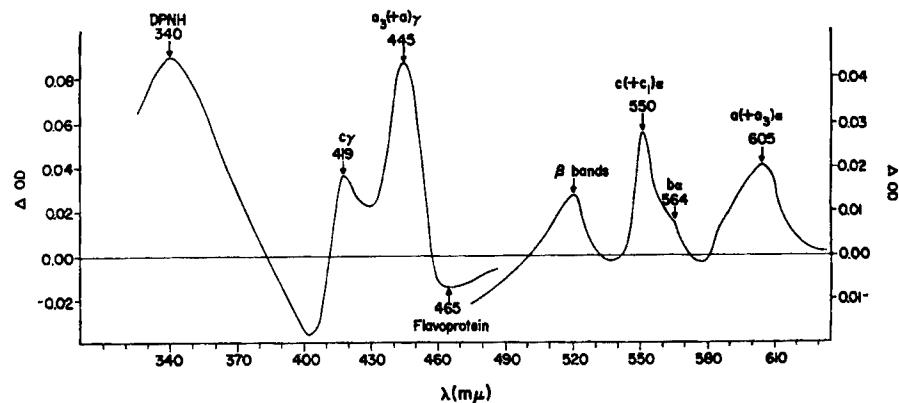


Figure 2 The difference spectrum of two excised toad sartorius muscles, reduced minus oxidized. Cytochrome *c* oxidase contributes in the alpha region at 605 nm mainly by heme *a* and in the gamma region at 445 nm about equally by hemes *a* and *a*₃. (DPNH = NADH and mμ = nm)

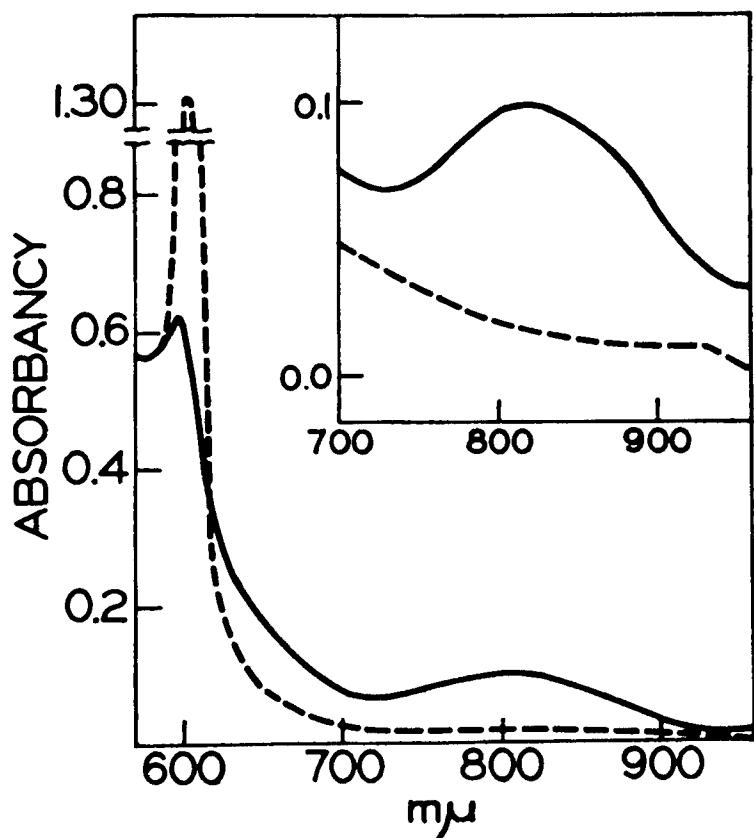


Figure 3 The NIR and alpha bands of cyt aa₃ shown as two absolute spectra; solid line oxidized, broken line reduced. (From 17)

minimize the wavelength dependence of light scattering intensity.⁸ This study showed that *in vivo* monitoring of the enzyme is possible and also showed that heme *a* is more reduced in the normally circulated brain than would be expected from the *in vitro* results.

Near Infra-Red Spectroscopy *in vivo*

This and other *in vivo* vs *in vitro* disparities demanded a more thorough investigation of their reality or proof of their artefactual nature. Mainly and very importantly heme *a* was much more reduced *in situ* than it should be when test tube data were extrapolated to intact tissue. This was the case for a number of tissues such as the gastric mucosa, intestinal mucosa, the kidney and even somewhat for the heart although not for skeletal muscle, at least not measurably so. These observations included several members of the chain that could be monitored in excised, hemoglobin-free tissues. But most significantly the heme *a* redox steady state observed with circulation intact, forced the resolve to look at all the components of the *aa₃* complex. Preliminary observations on the 445 nm peak showed much interference from the very strong hemoglobin bands in that region and made it clear that the *a₃* band presented considerable technical problems. It was decided to concentrate first on the NIR absorption band of the copper atoms shown in Figure 3.

The infra red region of the spectrum is infamous among hard working spectroscopists for the prevalence of intense absorption bands of water throughout most of the wavelength region. This precludes use of the IR for almost all spectrophotome-

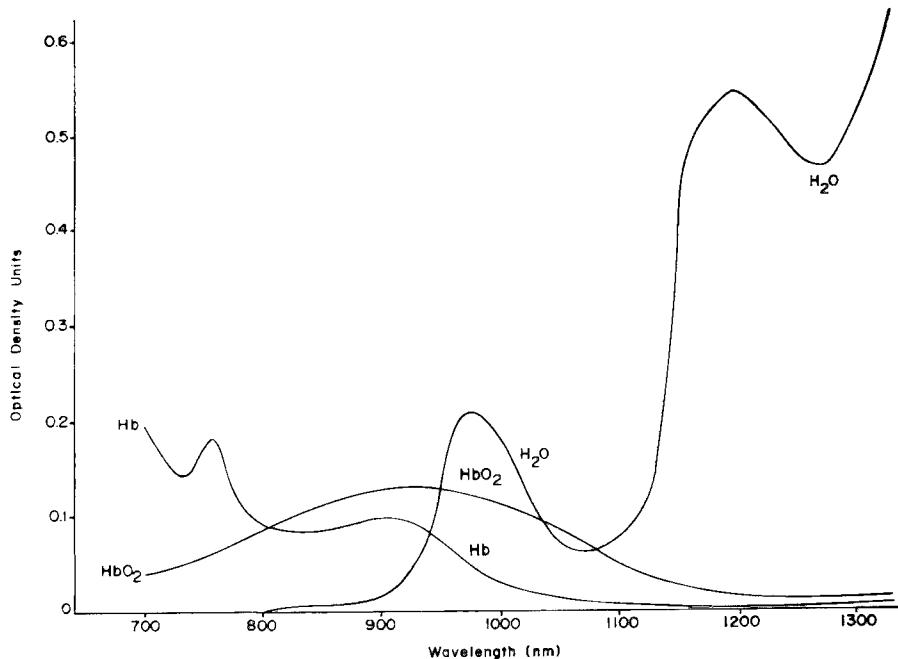


Figure 4 Spectra of water, oxy- and de-oxyhemoglobin in the NIR region. The latter two are shown in approximate proportion to the cerebral water content if all the blood in a normoxic brain were either Hb or HbO₂.

try of live biological materials. The exception is the Near Infra-Red from 700 to about 1300 nm in which the water bands range from absent to manageable. But beyond, water draws a curtain across this IR window.

By great good fortune the available range features absorption bands of hemoglobin and cyt aa_3 . An important aspect of the NIR for applications to hypoxia is the scarcity of biochemical compounds with absorption bands in this region of the spectrum. Little confusion exists concerning the assignment of absorption bands to different biochemical species. But for our purpose the most endearing NIR feature is the fact that of the species that do absorb, only Hemoglobin (and Myoglobin) and cytochrome aa_3 show changes in absorption patterns related to hypoxia. In Figure 4 the relative absorption strengths of hemoglobin and of water are depicted in an approximation of their relative strengths for the amount of blood within the brain under normoxic conditions. The calculation is somewhat unrealistic in so far that equal amounts of Hb and HbO₂ were assumed.

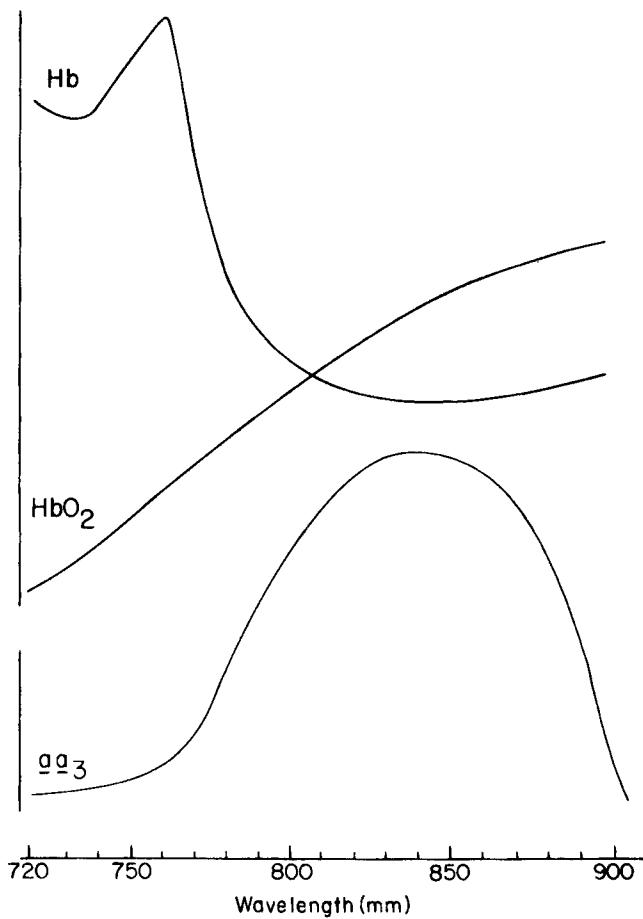


Figure 5 Absorption spectra (not to scale) of Hb, HbO₂ and cyt aa₃ in the NIR. Variability of the cerebral blood content by hypoxia or hypo/hypercapnia results in great variability of the relative contributions of the three.

In Figure 5 the three relevant absorption bands are shown in some detail: the Hb and HbO bands speak for themselves but the cyt aa_3 spectrum pertains only to the oxidized form, the reduced form does not have a distinct pattern in this region. The aa_3 spectrum shown is actually a difference spectrum: oxidized minus reduced. It should be noted first that the absence of an Absorbancy (Optical Density) scale is not an oversight. The amount of hemoglobin in the brain is quite variable due to vasoconstriction and vasodilatation caused by hyperoxia, hypoxia, hypercarbia or hypocarbia or due to adaptation to anemia or polycythemia. It could therefore be misleading to put an Absorbancy scale to the figure, suggesting a fixed relationship between cerebral hemoglobin and cytochrome *c* oxidase absorption. It can be stated however that the absorption of the summed hemoglobin components at the isobestic point (815 nm) relative to that of cyt aa_3 exceeds the latter fourfold to twentyfold depending on physiologic or pathologic vascular conditions. Although at first sight it would seem that the regional blood O_2 saturation could be determined without much jeopardy, in certain non-predictable cases an error of 25% could be introduced by neglecting the spectral contributions of cyt aa_3 .

In addition, until now it has not been possible to determine the concentrations of the absorbing molecules in such a highly light scattering medium as the intact brain, although several claims have been made. Since the photons emerge after being scattered multiple times on their way from the input point to the detector, the pathlength they have traversed is indeterminate. And knowledge of the pathlength is required to calculate the concentration of the absorber in the medium; the longer the pathlength as well as the higher the concentration, the greater the chance of a photon being absorbed. The intensity of light absorption does, however, reflect the amount of the absorber(s) encountered, regardless of pathlength.

As noted before, cytochrome aa_3 shows *in vitro* a strong affinity for oxygen: it has a P_{50} (50%/50% oxidized/reduced) of about 0.1 mmHg; above 1.0 mm Hg it is completely oxidized. Under physiologic conditions the P_{50} 's of myoglobin and hemoglobin are about 3-7 and 25-29 mm Hg respectively. This sequence of affinities fits nicely into our considerations of O_2 source-to-sink relationships, at least in muscle tissue. However it is our conclusion that, *in vivo*, this relationship does not hold in many tissues specialized for ion transport (central and peripheral nervous tissue, kidney, intestine, gastric mucosa) rather than for contractility. In our hands cytochrome aa_3 is much more highly reduced in the intact brain than would be expected from the test tube data.

This leads to two secondary hypotheses, i.e. (1) that an additional energy conservation point exists at the level of the $a_3 - O_2$ reaction and (2) that it serves the transport of ions.

Clearly it is not a simple task to strip the contributions of hemoglobin out of the cyt aa_3 signal. This has been done by experimentation combining observations on blood in the intact brain of deceased animals, on dense, strongly scattering suspensions of mitochondria and on intact rat and cat preparations with normal circulation or after exchanging the blood with artificial, fluorocarbon 'blood' (to a hematocrit < 1.0%) during O_2 ventilation at 1 atmosphere and with hyperbaric O_2 at 3 atmosphere and with selected respiratory chain inhibitors such as cyanide, carbon monoxide, amytal, antimycin A, etc.⁹ From this type of information algorithms were constructed that separate the absorption contributions of the hemoglobins and cyt aa_3 in the NIR region.

It should be stressed that scattering produces skewing of absorption maxima towards lower wavelengths. Since lower wavelengths scatter light more readily, these photons tend to be scattered more frequently and thereby will travel a longer path in the tissue and are thus more liable to be absorbed. Since scattering of light by tissues is hard to describe definitively for diverse tissues, various wavelengths and different optical arrangements, it is imperative to employ absorption co-efficients determined *in situ*, i.e. on the intact organ to be monitored, in our case the brain observed through scalp and skull. We have constructed our algorithms with these co-efficients. It is most important to emphasize that any algorithm must be tested *in situ* for the accurate identification of the intended signals and for the absence of cross talk between the signals. The best way of testing for the former is by the use of selected inhibitors of the respiratory chain. Cyanide blocks at the level of heme a_3 which should leave the copper moieties reduced. Inhibitors of the middle components of the chain should leave cyt aa₃ oxidized. A good test of cross talk in the aa₃ algorithm is provided by replacing most or all of the blood by a hemoglobin-free fluorocarbon suspension while ventilating the animal with oxygen. During this exchange of blood the aa₃ trace should not fall whereas a large or complete loss of Hb and HbO₂ is registered. However a loss of oxidized aa₃ should occur when the O₂ is decreased or replaced by N₂.¹⁴ Our algorithms have passed these tests.

The principles of an NIRS instrument are given in Figure 6; we have dubbed it the NIROSCOPE, short for Near Infra Red Oxygen Sufficiency scope. The diagram is of a somewhat superseded model but it does illustrate the salient points. Light from laser diodes is conveyed to the head and entered through the scalp. With neonates it is detected at the contralateral side, as shown. In adults the head tends to be too large for transillumination and a reflectance mode is used with the detector as far away as possible from the entry point, at least 4.5 cm, preferably more than 5 cm (Fig. 7). Light pressure of the end assemblies of the two probes (the "optrodes") eliminates the blood from the skin at the points of entry and detection. A large fraction of the photons that are entered is back scattered by skin and bone, away from the head. This light carries no cerebral information but fortunately dies out rapidly in logarithmic fashion with distance from the input point. The part of the light that enters the brain does so in a diffuse manner, since the skin's multi-layered nature destroys any collimation that might be present. It penetrates the gray matter readily but is reflected by the myelinated white matter; one of Mother Nature's best light scattering materials—which therefore appears white when viewed in white light.

The NIR photons travel various distances through the gray matter before emerging through skull and scalp. The farther the input and pickup points are separated the more cerebral information is carried by the light, i.e. the less the fraction of photons that have 'seen' only skin and bone. Since these tissues have low oxidative metabolic rates their aa₃ titer is accordingly low, i.e. not measurable. However the skin can contain a considerable and highly variable amount of blood. For this reason observations close to the point of light entry should be avoided since they predominantly carry information regarding blood in the scalp not in the cerebral circulation. Although light pressure eliminates skin blood from the points of input and detection, some light emanating from the bone between those two points will be turned back through the skull and thereby contribute to the signal observed at the point of detection. However, one saving grace for the NIR monitoring technique is the fact that the aa₃ signal can only be generated by cerebral tissue. And because of

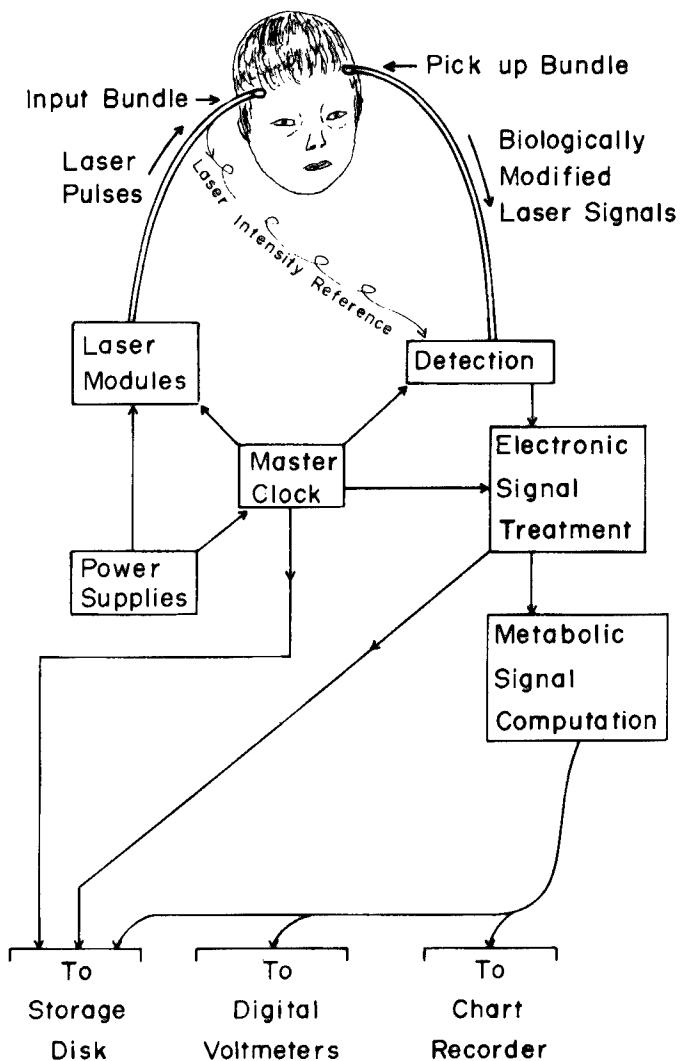


Figure 6 The Niroscope™ monitoring system depicted in the transillumination mode. The presently preferred display is by means of a laptop personal computer with active matrix color screen.

the paucity of mitochondria in glial cells, the signal originates specifically from the neurons.

Algorithm development

In order to identify the absorption contribution of cyt aa₃, algorithms must be constructed that strip the Hb and HbO₂ contributions out of the aa₃ signal. Once that is accomplished, we have also obtained the 'blood signals' as a byproduct useful in giving ancillary information for the better understanding of the causes of the aa₃ reactions.

Algorithm construction using *in situ* absorption coefficients starts in the form of simultaneous equations each summing the absorptions by the absorbers at a particu-

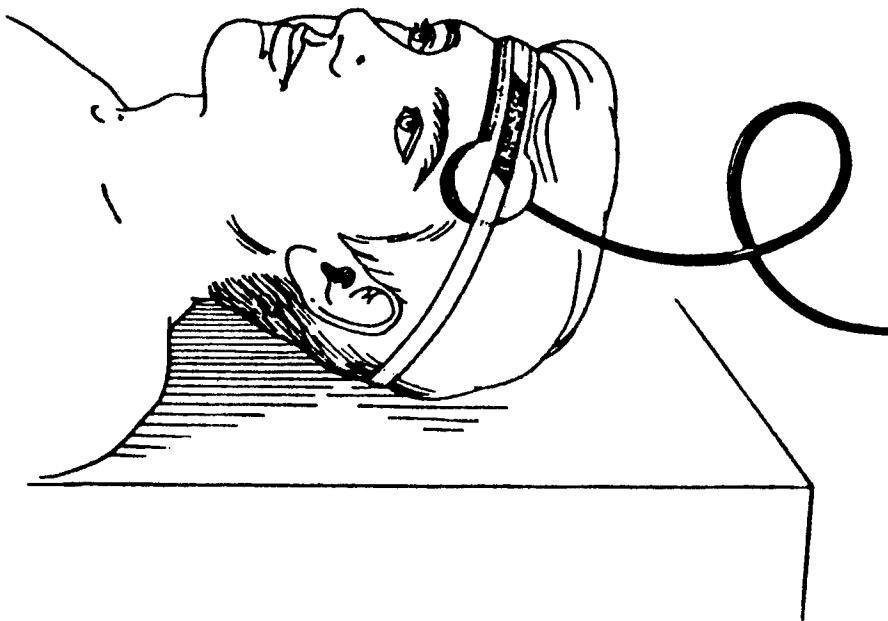


Figure 7 Sketch of the application of the headband used in the reflectance mode.

lar wavelength. This set of simultaneous equations is then solved by matrix inversion for the contribution of each absorber.⁶ There are a number of do's and don'ts in that exercise, nine in total. Since I can't seem to keep more than three thoughts in mind at a time, I have arranged them in three sets of three.

A) **Three cardinal conditions must be met** in the construction of NIRS algorithms for non-invasive monitoring.

A1.) The number of wavelengths must equal or exceed the number of absorbing molecular species to be distinguished; it takes a minimum of three absorption equations to solve for three unknowns: Hb, HbO₂ and cyt aa₃.

A2.) At least one wavelength may usefully be added to compensate for wavelength-dependent scattering effects and/or to provide some redundancy; more if the wavelength range covered by the light sources is wide &/or is in the shortest regions of the NIR, < 775 nm. In addition there is a small O₂-sensitive band (max at 865-870 nm) within the larger absorption band centered at 830 nm approximately.¹¹ This unidentified absorption band behaves biphasically, i.e. decreasing in hypoxia and then increasing in strength at final anoxia (FFJ- ν/d V, unpublished). In our hands a total of four wavelengths proved to be required for our purpose of on-line monitoring including the transition to death.

A3.) Each wavelength band must be narrow, i.e. as 'monochromatic' as possible. The absorption bands of Hb and HbO₂ rise or fall steeply over the wavelength scale which produces problems with light sources providing a wide bandwidth, such as the 40-50 nm half bandwidth typical of LED's. As the Hb content rises, say with hypoxia, light at 760 nm will be more attenuated than light at 800-810 nm and the midpoint of the emerging light will have shifted significantly. This will disturb the relative absorption co-efficients among the absorbers. These new prevalent wavelengths would require a new set of absorption equations and algorithms. Laser

diodes with bandwidths of 1-2 nm do not suffer this deterioration of the relative absorption strengths and are therefore to be preferred over LED's or even optical filters with typical bandwidths of 10-20 nm.

B) There are **three caveats to make NIRS more practicable** and the calculated biological signals more reliable. These are:

B1.) We must keep the light intensity to a safe and comfortable level by choosing a minimum number of wavelengths, each with maximal information content. This selection of a minimum number of wavelengths is in contrast with much benchtop spectrophotometry in which a wide band of 'white' light is presented for 'thumb print' analysis of a many banded absorption spectrum. For NIR monitoring in biological tissues we are blessed by the relative paucity of oxygen-dependent absorption bands.

B2.) The light intensity must be sufficiently strong to provide a viable signal at about 5 cm or farther from the input point on the head. At shorter distances the light detected during monitoring in the reflectance mode contains too large a signal fraction derived from the blood in the skin between the input and pickup points.

B3.) Another caveat is the need to use wavelengths that a.) are as near as possible to each other in order to minimize wavelength-dependent scattering distortions of the absorption bands but b.) still possess significantly different absorption coefficients of the components to be isolated spectrophotometrically.

C) This leaves us with **three strong, practical suggestions** for useful monitoring.

C1) The optical properties of the 'optrodes' and the skin should be matched in index of refraction as best possible. This can be done by applying a small amount of silicone optical gel to the optrode surface to form a matching interface between the two materials. Not only does this decrease the amount of light turned back at the air interfaces but is especially important in protecting against motion artefacts as the uncoupled surfaces move in respect to each other.

C2) Hair tends to interfere both by shading, especially if of a darker hue, and by creating multiple air pockets in which the above mentioned mismatch of refractive indices is maximized. Shaving may be indicated or, if the hair is not too thick, a larger amount of coupling gel can be helpful.

C3) The optrodes should exert sufficient pressure on the skin to expel its blood supply. In the case of cerebral monitoring this is easily accomplished by light pressure on the scalp. For transcutaneous monitoring of skeletal muscle the pressure has to be increased to be effective but the absence of bone below the skin also decreases the perpendicular reflectance. This allows more light to penetrate into the muscle tissue in which the much larger amount of Hb plus Mb overwhelms the skin contribution. For both types of monitoring we favor an elastic bandage to provide the pressure on the optrodes.

Recently a two-wavelength instrument was introduced into ICU and OR monitoring that violates at least five of these nine rules, caveats and suggestions; most flagrantly the need for more than two wavelengths to solve for three or more unknowns. Results obtained with it range from suspect to less than satisfactory and through unbelievable to hilarious. Any semblance of success in monitoring cerebral blood oxygenation with this instrument is probably obtained in cases when the scalp oxygenation happens to track the expected cerebral saturation, more or less. This notion is corroborated by the fact that upon death the instrument may not show a

major decrease in the oxygenation signal. This can be expected for blood in the skin with its low oxidative metabolic rate but not from blood in the cerebral cortex.

One of the more amusing articles on NIRS measurements with this instrument claims that corpses, pumpkins and other unlikely round objects can give readings of 80% oxygen saturation of the blood.¹⁵ This finding is just as silly as any attempt to use only two absorption equations to solve for two unknowns when several other unknowns are known to be present. But this most unfortunate situation must be corrected before NIRS monitoring will see widespread adoption. Our attempt at providing a correct signal of the cerebral blood oxygen saturation is described below in the last section.

NIRS Monitoring of the Cytochrome Redox State

With the Niroscope equipment it is possible to monitor smaller heads (rats, rabbits, neonate piglets and human babies) by transillumination (Fig. 6) and larger ones such as the adult pig's or human heads by reflectance (Fig. 7). Most of the signals that can be acquired are limited to the trends from the original baseline, i.e. set at the beginning of the monitoring session. Thus it is possible to monitor the changes in the aa₃ redox state, designated usually as Δaa_3 . In addition we can monitor the changes in the cerebral hemoglobins (ΔHbO_2 and ΔHb), and their sum, designated as change in blood volume (ΔBV) or more correctly in total hemoglobin (ΔtHb). These trends are therefore deviations from the condition at the start of monitoring. In the operating room the baseline can be set before anesthesia and is therefore a good Plimsoll mark throughout the surgical procedure. In ICU monitoring the patient's condition must be derived from other signs; effectiveness of treatment can then be judged by the changes from baseline. Because of the uncertainty of photon pathlength through the tissue, absolute concentrations can as yet not be determined on-line but such a capability for the Hb and HbO₂ concentrations will soon be in development if the NIH smiles on our request. Only recently has it become possible to acquire a reliable diagnostic value of the percent oxygenation of the blood in the cerebral circulation as will be shown further below.

Figure 8 shows the traces obtained while monitoring a rabbit's head during manipulation of the FiO₂ and FiCO₂. The expected changes in aa₃ oxidation and hemoglobin oxygenation are observed, including the vasodilatation by hypoxia. (It might be noted here that the rabbit's responses to hypercarbia are muted compared to those of other species, possibly an adaptation to spending much time in badly ventilated burrows.) Another, more remarkable finding is that the cytochrome aa₃ signal is quite responsive to the changes in FiO₂ immediately upon the decrease in HbO rather than being cushioned by its strong affinity for oxygen exhibited *in vitro*. From those data it would be expected that aa₃ would not become more reduced until tissue hypoxia had reached a rather extreme level.

The results from a cat experiment with a more extensive set of ventilatory gases are shown in Figure 9. The relationship between the optically observed amount of cerebral HbO₂ and the oxidized aa₃ signal are plotted on a normalized scale. Maximal oxidation/oxygenation was achieved by ventilation with a gas mixture of 95% O₂ plus 5% CO₂. Although this mixture does not fully oxidize and oxygenate, hyperbaric experiments with O₂ plus 5% CO₂ at 3-4 Atmospheres have shown that 95/5 at one atmosphere achieves better than 90% of that goal. Note that in normoxia (FiO₂ 0.21) both aa₃ oxidation and HbO₂ are at about 50% of their ranges.

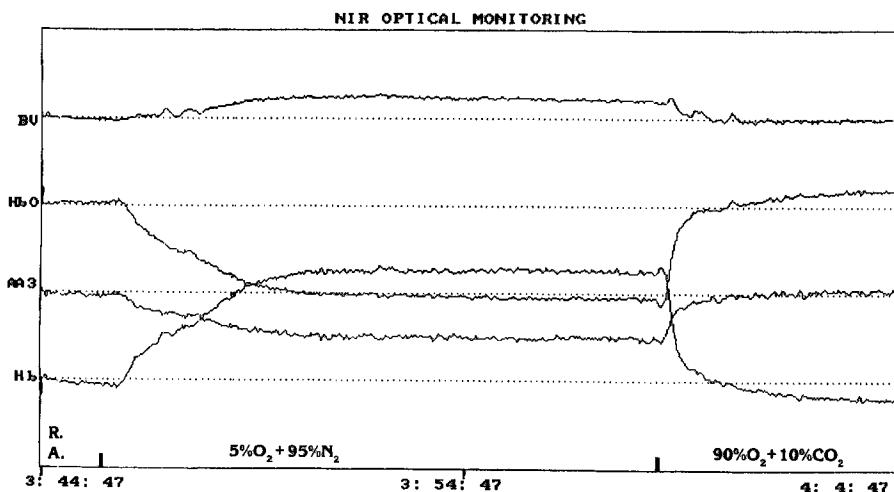


Figure 8 Niroscope traces from a rabbit's head, obtained by transillumination, in response to ventilation with Room Air; 5% O₂ (balance N₂); and 90% O₂ plus 10% CO₂. (The time base refers to the start of the experiment.)

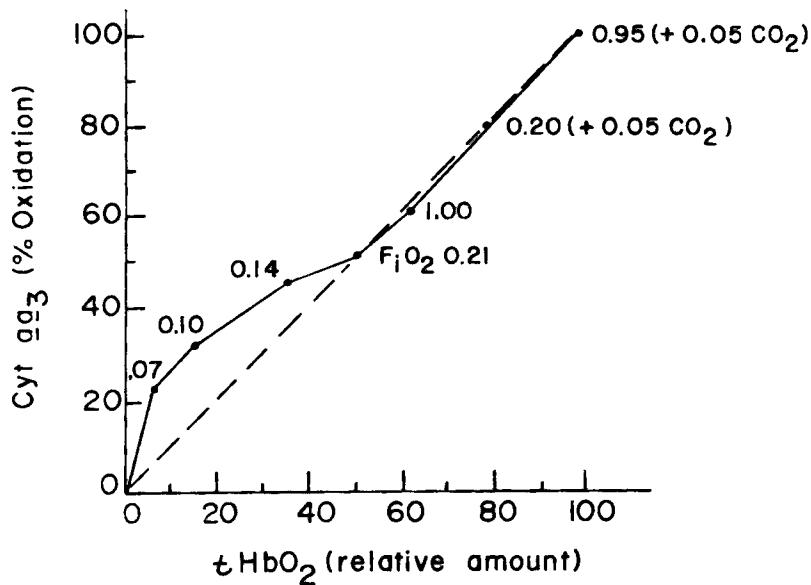


Figure 9 Correlation between the traces of cerebral HbO₂ and oxidized cyt aa₃ copper in a feline head. The numbers next to the data points refer to the F_iO₂.

Reaching the maximal amount of HbO₂ is aided by a strong vasodilatation due to hypercarbia; maximal aa₃ oxidation means that in this steady state no significant amount of the reduced form exists.

If the enzyme's affinity for O₂ were as strong as expected from the test tube data, aa₃ should have remained highly oxidized till much lower HbO₂ levels were reached. In other words the curve should have proceeded from the area at top right more or

less horizontally to low HbO_2 levels and then precipitously descended toward zero oxidized aa_3 when the HbO_2 values also trended close to zero. Instead we find a continuous parallel decrease in both signals until normoxia is reached. Then the aa_3 signal starts to deviate in the direction expected for a source-sink relationship. Thus cyt aa_3 is shown, only at lower tissue PO_2 levels, to be more avid for oxygen than the hemoglobin.

A first response to this finding would be the suspicion that the HbO signal is not sufficiently cleared out of the aa_3 signal. We have performed several types of experiments to test this notion—all showing that our algorithms do separate the two signals. The most direct and therefore most convincing were studies showing that blood could be replaced by colorless artificial blood without changing the aa_3 signal while the HbO_2 signal fell toward zero.¹³ Only in hypoxia and anoxia did the aa_3 signal show the expected loss of oxidized aa_3 .

Our conclusion is that there is a double effect. 1) Oxygen at higher than normoxic tissue PO_2 levels induces an oxidation of the copper of aa_3 in parallel with the oxygen availability from oxyhemoglobin. It has the appearance of an adaptation to higher tissue PO_2 levels. 2) At lower than normoxic PO_2 the expected higher affinity of aa_3 begins to show in a more conventional source-sink relationship, but still superimposed on the diagonal relation shown by itself at PO_2 levels above normoxia. It might be added that this level of reduction in normoxia agrees with the findings on heme *a* studied in various intact preparations.¹⁴

It should be noted that other instruments in other laboratories do not show this aa_3 behavior, at least not in outspoken fashion. However our algorithms are the only ones based on absorption co-efficients obtained *in situ* rather than from purified preparations studied *in vitro*.¹² We are more comfortable with these *ad hoc* co-efficients, with the *in vivo* tests of their efficacy in separating the signals, with the parallelism between the aa_3 NIR response and the heme *a* VIS response and therefore also with the concept of a labile aa_3 redox steady state responsive to the PO_2 of the tissue.

Corroboration of our viewpoint that aa_3 ‘adapts’ in affinity for oxygen comes from a recent study of the behavior of aa_3 in hemoglobin-free, fluorocarbon perfused cats.¹⁶ No cross talk between the aa_3 and the hemoglobin signals can therefore be present. This preparation showed a consistent reduction of aa_3 with falling tissue PO_2 , measured with O_2 electrodes, well before the cerebral oxygen consumption fell. This finding can be said to show that the enzyme is ‘adapting’ to the lower PO_2 . However from a reaction kinetics viewpoint it can also be regarded differently, i.e. that the increase of one reactant (reduced aa_3) will tend to compensate for a decrease in the other reactant (oxygen) and thus ‘cushion’ the reaction rate. No matter which explanation does prevail, *in vivo* the redox state of the enzyme varies under physiological conditions of O_2 availability.

The concept of a critical PO_2 .

This brings us to the need to address the oft asked question: “What is the critical PO_2 ?” I presume it to mean: at what low PaO_2 does oxygen first become limiting to the function and/or survival of cells; in our particular enquiry, the neurons. We first tried to answer it by observing members of the respiratory chain *in situ* primarily NADH and heme *a* of cyt aa_3 but we did not come up with a clear answer.¹⁴ Our studies resulted only in more questions “How to define it? And how can we measure it?” Not particularly clarifying insights!

However these early studies did show that the respiratory chain, studied *in situ* with Visible and Near Ultraviolet light, is more reduced than it has any right or reason to be in view of the high O₂ affinity determined *in vitro*. The most obvious difference was noticed in the steady state redox level of heme *a*. This led directly to the development of NIRS monitoring. The results were practically identical: the copper moiety of the aa₃ complex is also highly reduced under normoxic conditions, although not to the same degree as heme *a*. And the copper signal also responds with an oxidation to increases in PaO₂. The complex does seem to be adapting to lower tissue oxygenation or at least to accommodate the change by a changing redox level.

The upshot of these results is that the answer to the question "What is the critical PO₂?" is a resounding: "We don't know." and a timid: "Perhaps there isn't one?" or "It may depend on the circumstances such as time allowed for the system to adapt." Of course adaptation to a hypoxic environment is not such a foreign notion to people studying Mountain Medicine.

The regional Cerebral Blood Oxygen Saturation (rCBOS)

Trend monitoring is a satisfactory means with which to follow cerebral oxygenation especially so in the operating room when patients can be baselined with the Niroscope before induction of anesthesia. It is different under circumstances when the animal preparation must be anesthetized beforehand or when the patient is unconscious or in a compromised state for cerebral oxygen sufficiency. However, trend monitoring NIRS is extremely useful in the ICU as an early warning system of worsening life signs and as a means to gage the efficacy of medication or treatment on line.

In order to provide a more diagnostically valuable signal, we have recently completed tests of the % oxygen saturation determined with optical signals from the cerebral blood. This approach uses radically different algorithms which calculate this parameter on line with second-to-second updates. It is displayed as a bar graph on the screen and it is given as a number in the margin. We have dubbed this signal the regional Cerebral Blood Oxygen Saturation, abbreviated the rCBOS. It differs importantly from the pulse oximeter reading. Whereas the latter provides information only on the oxygenation of the arterial blood, the rCBOS pertains to the entire mix of arterial, venous and capillary fractions with emphasis on the microcirculation. In the reflectance mode it provides information about blood in the local, i.e. mostly the cortical, vasculature. In the trans-illumination mode the signal pertains not only to the regions in the direct line of transmission but, because of photon scattering, also to a large part, if not most of these smaller brains.

Since there are no previous determinations of an rCBOS parameter we are in the process of producing a data base for it. On an anecdotal basis it can be stated that measurements through the forehead of the senior author fall in the 68-69% range, whereas the junior author's forebrain consistently shows a half dozen percentage points higher oxygenation of his cerebral blood. Of course the question debated between us is whether the difference arises from better circulation or from more intense oxygen utilization.

In order to provide verification or rejection of the correct nature of the rCBOS signal we devised experimentation to sample the cortical venous blood from a sagittal sinus (SS) cannula using a young pig model monitored in the reflectance mode. In these experiments the temperature was lowered to 18°C while the experimental

animal was perfused by a cardio-pulmonary bypass technique. The low temperature was chosen to minimize the A-V differences. These experiments have shown a close adherence of the O_2 saturation determined from the blood samples and the rCBOS

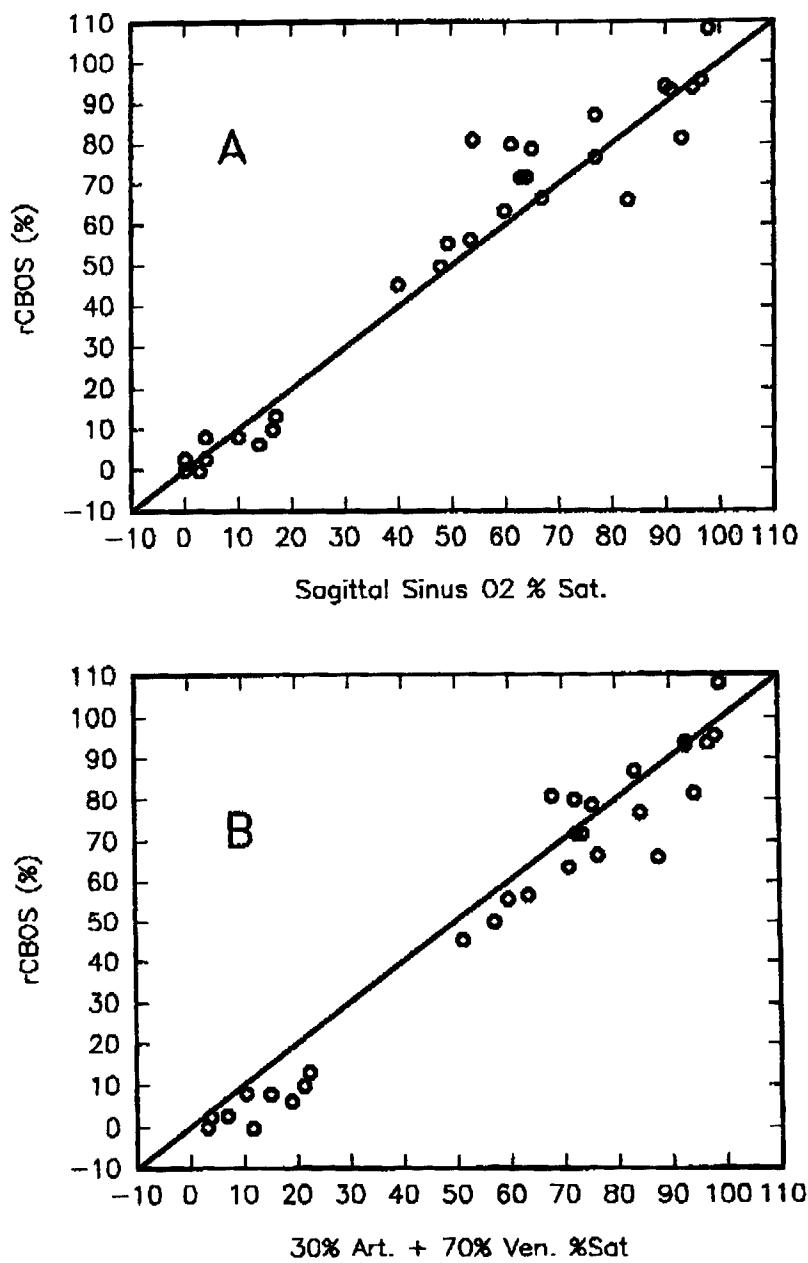


Figure 10 The regional Cerebral Blood Oxygen Saturation (rCBOS) as a function of the Sagittal Sinus % O_2 Saturation (top) and of the 30%-70% arterial to venous blood distribution in the brain. Two swine experiments at 37°C using the reflectance mode.

data over the entire range from 0% to 100% O₂. For a total of forty two determinations at various pump FiO₂'s on five preparations the bivariate correlation co-efficient was 0.98, proving the algorithm constructed for the optical rCBOS parameter to be satisfactory. The spread of the data between arterial and sagittal sinus (SS) venous oxygenation was small and little or no difference was seen between plotting the rCBOS data vs % O₂ Sat data from blood sample analyses weighted 30%-70% for arterial and SS values according to estimates of the distribution of arterial and venous blood in the cerebrum.

Only a few experiments have till now been performed at body temperature with normal circulation. In this protocol the anesthetized animal is ventilated with various FiO₂ mixtures including 95%/5% O₂/CO₂ and pure nitrogen. The rCBOS results from two experiments are shown in Figure 10. In plot A they are correlated with pure sagittal sinus blood, in B with 30/70 A/V weighting. A bit to our surprise we found that there is no clear difference between the two plots; actually the SS plot looks somewhat better upon visual inspection. More data are needed to perform a statistical analysis. If better agreement with SS blood than with a 30/70 A/V mix is shown, it would prove that either the NIRS reflectance mode favors cortical venous signals to a greater extent than the 30-70% assignment for the whole brain, or that in the optically monitored cortex the venous compartment is somewhat more predominant than in the whole brain.

SUMMARY AND CONCLUSION

The following summary of the case for Near Infra-Red monitoring of oxygen sufficiency can be made.

— Four wavelengths are recommended for monitoring cerebral oxy- and de-oxy-hemoglobin and cytochrome *c* oxidase (Cyt aa₃) non-invasively through the scalp and skull.

— Hemoglobin signals provide useful ancillary information but at times are suspect because of possible contamination by blood in other tissues (skin and bone) whereas Cyt aa₃ is only present in measurable amounts in brain tissue.

— Accurate algorithms can be constructed to monitor the regional cerebral blood oxygen saturation (the rCBOS) and the trend of Cyt aa₃ oxidation despite recent doubts generated by the premature introduction of an inaccurate cerebral oximeter.

— *In situ* determined absorption co-efficients are required for the construction of accurate algorithms.

— If the rCBOS is to be measured the Cyt aa₃ contribution to the optical signals must be ascertained and subtracted in order to avoid errors that theoretically can amount to 25% in some pathological situations.

— It is possible to monitor Cyt aa₃ and hemo- and myoglobin oxygenation in muscle tissue but the latter two can not be separated because their NIR spectra are identical.

— Cyt aa₃ is the most basic and most critical entity to monitor for the assessment of appropriate cellular oxygen availability.

— The redox steady state of cytochrome *c* oxidase in the brain *in vivo* is more reduced and more labile to tissue oxygenation than would be expected from the *in vitro* data on this enzyme complex.

— We interpret the above finding as showing that Cyt aa₃ in intact brain tissue does not possess a fixed high affinity for oxygen for its catalytic reaction yet may bind oxygen avidly at the heme a₃ site even at low PO₂.

In conclusion it should be emphasized that the concept of a "critical PO₂" has as yet not been precisely defined and its existence has as yet not been proven. In fact the "adaptability" of the Cyt aa₃ redox state to cellular O₂ availability tends to make this a nebulous goal, though a most important one that can perhaps best be investigated by further non-invasive optical studies coupled to well defined parameters of cellular function.

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CHAPTER 11

DOES TRAINING AND/OR RESIDENCE AT ALTITUDE IMPROVE SEA-LEVEL MAXIMAL AEROBIC POWER AND ENDURANCE PERFORMANCE?

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Many coaches, trainers, and elite athletes presently believe that training and/or living at altitude provides a significant benefit for performing competitive events at sea level. This belief is manifested by training centers that are located at moderate altitudes and by hypobaric chambers used for athletic training. In 1992, F.W. Dick of the British Athletic Federation listed no less than 23 worldwide training sites at altitudes ranging from 1,550 to 2,880 m.¹¹ Recent articles in magazines, newspapers, and the World Wide Web tout the benefits of hypoxic training.³² Vinyl-enclosed, hypoxia-exercise rooms at athletic clubs have been recommended for athletic training as well as "acclimatizing" skiers before they depart for high-altitude escapades.^{5,26} The 'secret' East German hypobaric chamber that was hidden under a mountain of dirt since 1979 reinforced the belief that hypoxic training was beneficial.⁸ Considering this worldwide effort, is there a scientific basis for the proposition that altitude exposure can provide some benefit to performance at sea level? Why is there such a divergence in beliefs between coaches/trainers and exercise scientists concerning training at altitude for subsequent sea-level performance?

There is no question that training at altitude improves endurance performance at altitude. The question is whether altitude training confers an advantage for sea-level competitive events. Athletes and coaches believe that altitude training improves not only performance at altitude, but that it also enhances sea-level performance for endurance events. In addition, they believe that: (1) it reduces the recovery time between competitive sea-level events; and (2) exercise-enhancing metabolic and physiologic changes are evident in athletes who originated and trained in high-altitude parts of the world.

Many scientists have shown that residence at altitude, without training, results in an improved oxygen carrying capacity, a right-shifted oxygen dissociation curve,

and increased capillary density and mitochondrial number. In addition, exercise at altitude is associated with increased mobilization of free fatty acids and increased dependence on glucose. These altitude-induced effects are also observed in sea-level training situations. The combination of effects should give the altitude-trained athlete an advantage in sea-level competitions. The problem lies in developing the appropriate experimental hypothesis and design, and in overcoming difficulties in data collection.

Relating exercise performed at altitude on a laboratory treadmill or cycle ergometer to track and field competitions at altitude or sea level is most likely not going to be successful for reasons shown in Figure 1. If a subject's aerobic power ($\dot{V}O_2\text{max}$) does not improve, it may be concluded that altitude training had no effect. Unfortunately, many studies only report post-exposure, sea-level aerobic capacities on group data, rather than individual data. Usually, studies are not designed to test endurance performance after a training period involving altitude exposure.

Five study design considerations are listed in Figure 2. In any training program, training stimuli (intensity, duration, and frequency) should be increased as physical fitness improves, otherwise there will be no additional stimuli to elicit additional improvement. With acute altitude exposure, there are decreases in $\dot{V}O_2\text{max}$. Endurance performance for any exercise lasting longer than 2-3 minutes also suffers. Endurance training at altitude at the same absolute power output as at sea level significantly increases the relative exercise intensity and thus the difficulty of the task. Therefore, the training intensity at altitude is typically reduced. While at altitude, there is a distinct possibility that absolute training intensity will be below the level needed to improve fitness at sea level. An individual could fall into any of the experimental categories shown in Figure 2 and not increase aerobic power and endurance performance, relative to sea level control group values.

REASONS FOR LACK OF AGREEMENT

- Laboratory measures of performance may not reflect athletic performance
- Measures of aerobic capacity may miss small changes in anaerobic capacity that could affect performance
- Athletic skill, judgment and strategy are not reflected in laboratory measures
- Altitude training may be better for some athletes than for others - Reduced intensity leading to detraining
- Difficulty in experimental design

Figure 1 Five principal reasons for an inconsistency in thought about the efficacy of altitude training or residence on subsequent sea-level exercise performance.

EXPERIMENTAL DESIGN CONSIDERATIONS

- Absolute vs relative exercise intensity
- Work high - Live high (17 studies)
- Work high - Live low (6 studies)
- Work low - Live high (2 studies)
- Work low - Live low (Control)

Figure 2 Basic experimental study designs that should be considered in testing the hypothesis that altitude training and/or exercise affects subsequent sea-level performance. Three are variations of the work-live paradigm at sea level or altitude with a fourth as a sea-level control. Inherent in all the environmental designs is the decision to study relative versus absolute work intensities.

Of 23 different studies listed in Tables 1-3, the majority involve exercise training at high altitude with residence at high or low altitude. The study sampling encompasses altitudes ranging from 1,300 to 5,700 m, durations of 12 days to 19 weeks, and subject aerobic fitness levels of 37 to 74 ml·kg⁻¹·min⁻¹. Two additional studies,^{15,24} not shown in the Tables, are mentioned because they involve living at high altitude but working at low or sea-level elevations.

Table 1. Hypoxic Exercise with Altitude Acclimatization - 'Work high - Live high'

Ref #	Training Altitude	Duration	Fitness (ml·kg ⁻¹ ·min ⁻¹)	Control Group	VO ₂ max improvement at Altitude	on Return
(3)	2300 m	10 d	?	N	+ 6%	+ 8%
(22)	3800 m	5 wks	37	N	+ 4%	+ 14%
(7)	3475 m	20 d	44	N	0%	0%
(13)	2300 m	23 d	?	N	+ 2%	+ 9%
(27)	4300 m	12 d	48	N	0% (?)	n/a*
(21)	4300 m	16 d	51	N	+ 10%	n/a*
(6)	4000 m	55 d	63	N	+ 3% (ns)	0%
(29)	2270 m	4 wks	65	N	+ 5%	n/a
(31)	2250 m	19 d	>60?	N	+ 5%	n/a
(14)	23-4300 m	varied	>60	N	0%	0%
(9)	2300 m	alt. 1-2 wks	74	N	+ 2%	+ 4%
(12)	3090 m	17 d	72	N	+ 2%	+4%
(28)	2700 m	2 wks	72	N	0%	0%
(20)	4300 m	4 wks	38	Y	n/a	+ 10% n.s.**
(19)	3100 m	17 d	66-68	Y	0%	0%
(1)	2300 m	3 wks	73	Y	0%	0%
(33)	2100 m	2 wks	74	Y	n/a	0%

*17-60% increase in endurance time

** same as control

Work high - live high

Of 17 studies combining exercise and altitude acclimatization shown in Table 1, 13 studies measured $\dot{V}O_2\text{max}$ after return to sea level (upper 13 references). Nine of these studies had no comparable sea-level control group; seven studies reported increases in $\dot{V}O_2\text{max}$ at altitude or after return to sea level. Differences between studies in which subjects trained while residing at altitude, using and not using control groups, can be attributed to differences in training altitudes, training duration, and subject aerobic fitness levels. Only four (lower four references) of the 13 studies had sea-level control groups, and they reported changes in endurance performance and $\dot{V}O_2\text{max}$ that were the same or better than the altitude group; that is, none reported statistically significant increases in $\dot{V}O_2\text{max}$ during or after altitude training when compared to the control group. Therefore, any changes in $\dot{V}O_2\text{max}$ resulted from a training effect rather than altitude per se. Improvements in track and field events were reported on two studies, however, without changes in $\dot{V}O_2\text{max}$.

Table 2. Hypoxic Exercise Without Altitude Acclimatization - 'Work high - Live low'

Ref #	Training Altitude	Duration	Fitness (ml·kg ⁻¹ ·min ⁻¹)	Control Group	$\dot{V}O_2\text{max}$ improvement at Altitude	$\dot{V}O_2\text{max}$ improvement on Return
(4)	4200 m	19 wks	45	N	+ 14%	+ 36%
(25)	3050-4268 m	3 wks	54	Y	+ 10%	+ 10%
(30)	2250 m 3450 m	4 wks	46 46	Y Y	+ 8% + 14%	+ 18% + 10%
(23)	2500 m	5 wks	43	Y	+ 12-18%	+ 11-15%
(10)	4100-5700 m	3 wks	56	Y	+ 11%	0%
(34)	2300 m	4 wks	71	Y	0%	0%

Work high - live low

Six studies listed in Table 2 were examined with physical training at altitude or in hypoxic conditions for short periods with residence at sea level. Five of the six (lower five listed) studies had comparable sea-level control groups. The upper five studies reported improvements in $\dot{V}O_2\text{max}$ whether tested at sea level or under hypoxic conditions; the last study listed reported increases in exercise capacity, but not $\dot{V}O_2\text{max}$ in the altitude experimental group, but not the control group when tested only in hypoxia. Surprisingly, most studies report results consistent with a beneficial effect of hypoxic exercise training for subsequent performance in hypoxia or at sea level. Why living at sea level and training in hypoxia may be more beneficial than living and training at altitude may be related to changes in plasma and blood volumes as well as to changes in hematocrit and hemoglobin. Further studies are needed using this paradigm to determine whether beneficial effects result from intermittent hypoxic exercise.

Work low - Live high

Because of logistical and experimental obstacles, only two studies were found that investigate the problem of working at low altitude but living temporarily or permanently at high altitude. Levine et al.²⁴ tested the hypothesis that training at low altitude (1,200-1,000 m), but acclimatizing to moderate altitude (2,500 m) for 4

weeks will improve sea-level performance in well-trained runners. Thirty-nine subjects (12 were females) were divided into 3 groups: (1) living at high but training at low altitude; (2) living and training at high altitude; and (3) and living and training at low altitude. The primary measure of performance was a 5K run. The "live high-train low" group improved their sea-level 5K time by ~15 sec. Results were attributed to the combination of altitude acclimatization (5% increased red cell volume) and maintenance of training intensity at sea level.

The second study attempted to obviate the problem of training elevations, duration, acclimatization rates, and training intensity by having subjects train at altitude with complete acclimatization (i.e., altitude natives). Favier et al¹⁵ studied 30 sedentary altitude natives at 3600 m who cycled 30 min/day, 5 x/week for 6 weeks, after which they were randomly assigned to three groups and tested at: (1) 70% of altitude specific $\dot{V}O_2\text{max}$ with ambient air (control); (2) the same relative intensity with 31.4% O₂ (70% of normoxic $\dot{V}O_2\text{max}$); and (3) the same absolute intensity with 31.4% O₂ (70% hypoxic $\dot{V}O_2\text{max}$). Groups 2 and 3 were living high and working low; group 1 was the control. All three groups improved their $\dot{V}O_2\text{max}$ to the same extent. The authors' combined conclusion was that altitude acclimatization combined with "sea-level" training does not cause a greater improvement in $\dot{V}O_2\text{max}$ than in hypoxia alone.

Table 3 shows studies in which subjects resided and trained at altitude, but also participated in various athletic endurance events at sea level. On testing at sea level, either both $\dot{V}O_2\text{max}$ and endurance performance did not improve,^{2,6} both did improve,^{9,13} or $\dot{V}O_2\text{max}$ improved but endurance performance did not.^{16,28,33} Only two,^{2,33} of the seven studies had sea-level controls. The reasons for the lack of uniformity may lie in differences in exercise intensity, duration of altitude exposure, and possibly even in the degree of aerobic fitness prior to training. Another factor to consider is that small changes in $\dot{V}O_2\text{max}$ may be reported as statistically non-

Table 3. Training at Altitude for Sea-Level Performance

Ref #	Training Altitude	Fitness (ml·kg ⁻¹ ·min ⁻¹)	$\dot{V}O_2\text{max}$ improvement at Altitude	on Return	Endurance Performance
(13)	2300m	runners	+ 2% (ns)	+ 9%	880 yd, 1 and 2 mile
(6)	4000 m	63	+ 3% (ns)	+ 0%	None
(14)	2300 m	69	+ 4%	+ 1% (ns)	1,2, and 3 mile
	2300, 3100, 4300 m	68	0% (2300)	+ 1%	
	4300, 2300, 4300 m*	46	+13% (2300) 0% (4300)	+ 2%	
(9)	2300 m	74	+ 3-4%	+ 5%	1,2 mile, personal bests
(1)	2300 m*	73	+ 3% (ns)	-3% (ns)	None
(28)	2700 m	72	n/a	0%	17% increase short-term running
(33)	2000 m*	74	n/a	0%	None

* SL control group

significant, but these small changes can be associated with relatively large changes in endurance performance. A small, non-significant change in $\dot{V}O_2\text{max}$ of 1%-3% can be associated with a 12%-45% increase in endurance performance.^{17,18,27} Because of a "no change" result in $\dot{V}O_2\text{max}$, endurance tasks are often not evaluated. In addition, quite often only group data are reported, thereby missing individual improvements.

Summary

Research clearly shows that altitude training will improve altitude physical performance. However, research studies to date have not conclusively shown that sea-level performance will be improved with any kind of physical training at altitude. Since many of these studies did not have subjects compete in individualized competitive endurance events at sea level, it is difficult to state emphatically that athletic performance was improved as a result of altitude training. Maximal aerobic power, which is usually measured at sea level after an altitude exposure, may not be statistically different, but may result in realistic improvements for competitive athletes. Living at altitude but training at sea level allows exercise intensity to remain high, in addition to stimulating any beneficial responses that may result from sleeping at altitude. Finally, athletes may simply choose to train at altitude in any fashion that is convenient for the psychological advantage that it gives them.

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CHAPTER 12

THE DIAGNOSIS OF ACUTE MOUNTAIN SICKNESS IN PRE-VERBAL CHILDREN

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Introduction

The Lake Louise Scoring System (LLSS) defines acute mountain sickness (AMS) in adults but cannot be applied to pre-verbal children. The objective of this study was to establish the diagnostic criteria for AMS in pre-verbal children.

Methods

Children ≥ 3 and ≤ 36 months old from the Denver, CO area (1609 m, 5280 ft) without known cardiopulmonary disease or acute illness were studied over 4 separate days. A fussiness score (FS) was used as the headache equivalent score. The remainder of the LLSS was modified into a pediatric symptom score (PSS) assessing appetite, vomiting, playfulness and ability to nap. We defined the children's LLSS (CLLS) to be FS+PSS. Parents recorded the FS at 1100, 1300, 1500 & 1700 hours and PSS at 1500 hours of each study day. Days 1 & 2 were measurements at home; day 3 reflected travel without altitude change to Ft. Collins, CO (1615 m, 5300 ft); and day 4 involved travel to the Keystone, CO summit lodge (3488 m, 11,444 ft). On days 3 & 4 pulse oximetry (SpO_2), pulse (P), and respiratory rate (RR) were measured; and adults completed the LLSS.

Results

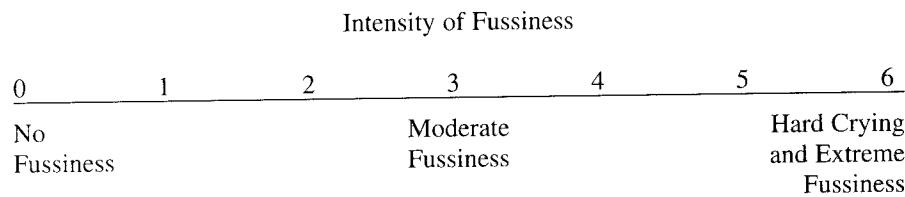
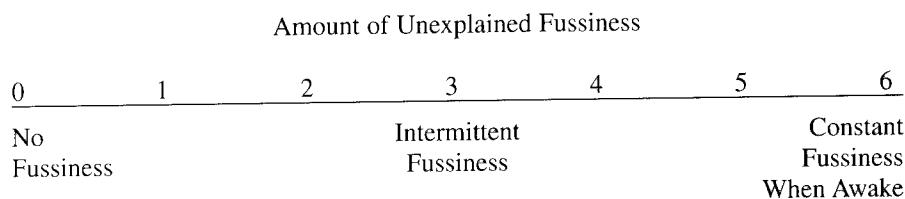
Twenty-three subjects (14 boys), ages 20.7 ± 9 mos. participated. The mean CLLS demonstrated no differences on days 1, 2, or 3. Two standard deviations from the mean were exceeded on days 1-3 if $\text{FS} \geq 4$ and $\text{PSS} \geq 3$. On day 4 five subjects (21.7%) had AMS (defined as a $\text{CLLS} \geq 7$) and these scores normalized 2 hours after descent. No differences existed between the subjects with or without AMS regarding SpO_2 , P and RR. Forty-five adults participated and 9 (20%) had AMS by the LLSS after 4 hours at altitude.

Conclusion

We define AMS in pre-verbal children as a $\text{CLLS} \geq 7$ with $\text{FS} \geq 4$ and $\text{PSS} \geq 3$, in the setting of recent altitude gain. The incidence of AMS in pre-verbal children (21.7%) was similar to adults (20%).

Fussiness Score

Fussiness is defined as a state of irritability that is not easily explained by a cause, such as hunger, teething or pain from an injury. Fussy behavior may include crying, restlessness or muscular tension. Please rate your child's typical fussy behavior over the last 2 hours without the benefit of your intervention.



TOTAL SCORE IS THE SUM OF EACH OF THE ABOVE COMPONENTS _____

Pediatric Symptom Score

Rate How Well Your Child Has Eaten Today

- 0 - normal
- 1 - slightly less than normal
- 2 - much less than normal
- 3 - vomiting or not eating

Rate How Playful Your Child Is Today

- 0 - normal
- 1 - playing slightly less
- 2 - playing much less than normal
- 3 - not playing

Rate Ability of Your Child to Nap Today

- 0 - normal
- 1 - slightly less or more than normal
- 2 - much less or more than normal
- 3 - not able to sleep

TOTAL SCORE IS THE SUM OF EACH OF THE ABOVE COMPONENTS _____

CHAPTER 13

USING ^1H -MRS TO EVALUATE THE CREATINE- PHOSPHOCREATINE POOL IN HUMAN MUSCLE

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Introduction

Over the last 25 years studies have been conducted on the rest-work-recovery transitions of muscle using ^{31}P -MRS.^{5,6,11} The implicit assumption in all of this work is that the creatine-phosphocreatine pool (Cr_{TOT}) accessible to creatine-phosphokinase (CPK) is constant in varying metabolic states; many studies also assume that the MRS (Magnetic Resonance Spectroscopy) visible PCr/Cr pool is not only fully accessible to CPK under all conditions, but that it is also the same as the chemically extractable pool of these two intermediates.^{3,6,11,14} Other workers hypothesize that the structural organization of muscle creates physical constraints, localized PCr/Cr pools, and possible preferred access pathways of PCr and Cr metabolism.^{2,4,7,8,12,13} These workers might expect that the Cr_{TOT} may not be constant under all metabolic states. In this study we used ^1H -MRS to examine the creatine pool. Since it is not possible to discriminate between PCr and Cr from the proton resonance at 3ppm, we reasoned that a single resonance line in principle should be utilizable for estimating the summed behaviour of Cr_{TOT} . We examined human gastrocnemius in three different conditions: at rest, in ischemic fatigue, and following attempted alteration of Cr_{TOT} by creatine supplementation. We found that the ^1H - Cr_{TOT} peak intensity and its transverse decay time, T_2' , are different in muscle in ischemic fatigue from muscle in its resting state. Observations of peak intensity and T_2' , were also made on the peak adjacent to Cr , and designated choline, Cho. However, conclusions drawn from these observations should be tempered by the realization that more than one resonance may reside under the Cho peak.

Methods

Study design: Experiments were carried out on 12 healthy power trained (PT) athletes (VO_2 47.65 ml/kg/min) and 12 healthy endurance trained (ET) athletes (VO_2 65.04 ml/kg/min). Subjects performed a familiarization exercise trial (similar to that previously reported⁵) returning after 24 hours for the initial experimental trial. Following this trial, 6 subjects from each group undertook a Cr loading regime for 7

days (5g doses at 4 times per day). The other 12 subjects took sucrose as a placebo. On the final day of loading, subjects returned to repeat the exercise protocol. Six of the subjects returned to complete another trial focused mainly on determining the T_2' for the proton NMR signals from the Cr and Cho region of the spectrum and for water.

Exercise Protocol: All subjects completed a plantar flexion exercise of the right foot against a load of 10 kg with a frequency of 1 stroke/s. The load was increased by 1kg/min until the subject attained volitional fatigue. A displacement transducer connected to the subject's heel provided for calculation of total work done. Immediately following exhaustion a pressure cuff was inflated for 5 min (>350 mmHg) superior to the knee to allow collection of spectra prior to PCr resynthesis. Upon removal of the cuff, recovery of Cr_{TOT} was assessed for 10 min. and the same measurements were collected.

MRS: The exercise was performed while lying supine in a 3 Tesla superconducting magnet with the right medial gastrocnemius centered in a circumscribing radio frequency coil. Guided by a proton image of the calf, a PRESS (Point Resolved Spectroscopy) sequence was used to resolve the 1H -visible Cr/PCr peak during rest and ischemic fatigue.¹ Cr_{TOT} intensity data were acquired at a standardized echo time (TE) of 100ms for 164 averages from a 4.5 cm^3 volume of interest (VOI) in the medial gastrocnemius. The TR in these experiments was 2 seconds. Representative spectra for muscle at rest and in fatigue (Figure 1) indicate a clear resolution of Cr_{TOT} from the nearby upfield peak (Cho, with possible contributions from carnosine and taurine). Subsequently the MRS evaluation was extended (with 6 subjects) to allow estimates of T_2' under the same exercise protocol. In this second study, relaxation data were acquired at TEs of 40, 60, 80, 100, 130, and 160 ms in the resting state and TEs of 40, 60, 80 ms in the ischemic fatigue state from the same volume of interest. The signal to noise ratio and the number of data points in the relax-

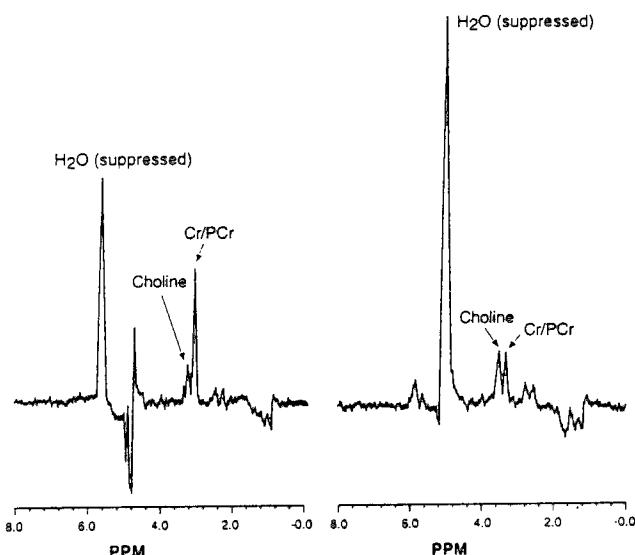


Figure 1 Representative *in vivo* 1H -MRS spectra recorded from the medial gastrocnemius showing from left to right, resting and ischemic fatigue Cr_{TOT} and choline intensities.

ation analysis, particularly in the state of ischemic fatigue, precluded a multicomponent analysis of the decay, although in the resting state it appeared to be largely single component.

Results:

When we compared resting muscle to muscle in ischemic fatigue, the two main observations that we made were (a) a decrease during fatigue in the Cr_{TOT} peak area (measured at $\text{TE} = 100$ ms) and (b) a simultaneous decrease in the T_2' calculated as described above. We then estimated the T_2' values for Cho from the same spectral data sets, and for water from spectra acquired in the two metabolic states. The T_2' for Cho, like that for Cr_{TOT} , appeared to decrease during fatigue, but the T_2' for water was unchanged in the fatigue state of muscle from that at rest (Table 1).

Table 1
Change in T_2 for various metabolites in the medial gastrocnemius
at rest and ischemic fatigue.

Metabolite	T_2 (ms) Rest	T_2 (ms) Ischemic Fatigue
Creatine (n = 30) (SE)	117 (4.1)	40 (6.5)
Choline (n = 6) (SE)	61 (4.3)	45 (2.4)
Water (n = 6) (SE)	30.3 (1.8)	32 (1.0)

Discussion:

Empirically our analysis demonstrates that during the transition from exercise to fatigue a decrease takes place in both the Cr_{TOT} intensity and in its T_2' . The observed decrease in Cr_{TOT} intensity was about 40%; however, when corrected for the change in T_2' , this change transforms into a 2-fold increase in estimated Cr_{TOT} intensity at $\text{TE} = 0$ ms (Table 2). It should be borne in mind that the magnitude of this estimate is quite sensitive to T_2' , but even so an increase in the Cr/PCr pool seems beyond doubt and is consistent with recent studies by Styles et. al.¹⁰

Possible causes of the change in T_2' and Cr_{TOT} intensity could include (a) an increase in deoxygenated (and therefore paramagnetic) myoglobin (Mb) in the intracellular compartment and in deoxygenated hemoglobin (Hb) within the microvasculature of the VOI, (b) a change in intracellular water content, or (c) a change in the intracellular milieu. Let us consider each of these in turn.

First, during initial rest and exercise, Mb and Hb are mostly saturated with oxygen and thus diamagnetic; with pressure cuff inflation at the end of exercise (ischemic fatigue), Mb and Hb become largely deoxygenated. An effect of paramagnetic Mb on the proton spectra of metabolites has recently been demonstrated.⁹ If Cr_{TOT} were to be affected this way, then one might expect all cytosolic metabo-

Table 2

Change in the peak intensities calculated at TE = 0 ms for various metabolites in the medial gastrocnemius at rest and ischemic fatigue.

Metabolite	Rest	Ischemic Fatigue
Creatine (n = 6) (SE)	1.21 (0.08)	2.17 (0.17)
Choline (n = 6) (SE)	1.58 (0.18)	4.16 (0.14)
Water (n = 3) (SE)	89.66 (9.81)	92.42 (9.6)

lites and water to respond similarly. In fact, the T_2' of water is the same in muscle at rest as in the fatigue state and the preliminary check on T_2' of the Cho peak suggests a modest 25% decrease. What is more, during recovery, Hb and Mb very rapidly reoxygenate during reperfusion and hyperemia; however, we found that more than 10 min. are required for the Cr_{TOT} intensity to return to the normal pre-exercise state (data not shown). Thus, at least tentatively we can conclude that deoxymyoglobin is not a major contributing cause of the observed Cr_{TOT} spectral changes.

Secondly, water shifts are well known during exercise and in our protocol a 5-15% increase in intracellular water content would be expected. This would tend to increase the T_2' of metabolites free in solution, so this parameter also does not seem to help explain the direction and magnitude of changes in Cr_{TOT} intensity or in T_2' .

Thus we are left with our third option: to account for the observed changes in muscle Cr_{TOT} spectra on transition from rest to an ischemic fatigue state it seems necessary to postulate changes in the intracellular environment, such as changes in viscosity or in binding states, either of which could alter both T_2' and peak intensity. At this time, there is no information available as to the nature of any such changes. It is interesting to note, however, that during ischemic fatigue Cr_{TOT} is composed largely of creatine; it may be reasonable to assume that change in creatine concentration and in its relative mobility are the main contributors to the observed changes in MRS spectral behaviour.

Conclusions

For physiologists and metabolic biochemists, the most interesting outcome of this study is the indication of an unexpected increase in estimated Cr_{TOT} in fatigued muscle compared to that in the resting state with a simultaneous decrease in its mobility (lower T_2'). These MRS results are not consistent with the assumption that the rules of solution chemistry apply globally to the total PCr/Cr pool. In fact they are more consistent with the concept that the behaviour of the Cr_{TOT} is constrained by intracellular structure and order in which the entire pool may not be accessible to CPK in all metabolic states.

For magnetic resonance spectroscopists the most interesting outcome is the indication of significant reductions during exercise in the T_2' for creatine and possibly also the Cho peak, but with little effect on the T_2' of water. Whereas the effect of

deoxyMb and deoxyHb on the relaxation behavior of metabolites *in vivo* supplies a possible partial explanation of our data, the kinetics of recovery and the effects of increased intracellular water concentrations are not consistent with this being a dominant cause of the observed changes in the muscle MRS spectra. Thus the detailed mechanisms giving rise to the quantitative differences between individual proton species remain to be resolved.

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CHAPTER 14

THE EFFECT OF GENDER ON VENTILATORY DRIVE, BODY TEMPERATURE, AND HYPOXIA TOLERANCE OF RATS

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Abstract

In mammals, acute exposure to hypoxia elicits beneficial responses such as increased ventilation and cardiac output. Hypoxia also induces hypothermia, which may also be beneficial by reducing O_2 demand and minimizing stress response when O_2 supply is limited. We found that female rats have a greater hypoxic ventilatory response than males and hypothesized that they would be more tolerant of hypoxia. We studied 60 female and 60 male Long-Evans rats. Minimitter® transmitters for body temperature (T_b) were implanted under general anesthesia (Nembutal®). Rats were exposed to 21, 16, 12, 10, 8, 6, 4, 2% O_2 (balance N_2) for 30 min each in chambers kept at 31° (clamped) or 20°C (hypothermic). The limit of tolerance was defined as the point at which animals became ataxic. Hypothermia significantly increased tolerance of rats although ventilatory response was blunted in both genders. Females were significantly more hypoxia tolerant than males. This was correlated with a lower T_b of females in the hypothermic group but not in the clamped group. To examine the role of estrogen, we tested the effect of ovariectomy and ovariectomy plus estrogen replacement (slow release pellet subcutaneous -0.5 mg 17-b estradiol in cholesterol). Ovariectomized rats showed significant loss of tolerance but did not attain male values. Replacement estrogen restored the tolerance. We conclude that two mechanisms, hypothermia and increased ventilation, increase tolerance of female rats to severe hypoxia.

Introduction

Imposed hypothermia is a well recognized clinical intervention during neuro and cardiac surgery (13 for review). In nature, the protective influence of hypothermia is also widely utilized, i.e., most species have a regulated, reversible, reduction in body temperature (T_b) when exposed to low ambient oxygen as well as other stresses that compromise oxygen transport. This response has been demonstrated in organisms ranging from protozoans to mammals (14 for review).

In rats, hypoxia elicits both increased ventilation and decreased T_b . The interaction between these two beneficial responses to hypoxia have previously been

studied.^{6,11} While most data are for male rats, a rapidly growing literature attests to gender differences in stress response. Female rats show enhanced ventilatory mechanics and response to hypoxia¹⁷ and reduced polycythemic and cardiopulmonary responses *in vivo* during chronic hypoxic exposure.¹⁰ This paper presents recent data showing that female rats have increased hypoxic ventilatory response³ and are more tolerant of severe hypoxia than male rats.¹⁵

Methods

All protocols and surgical procedures employed in this study and approved by the Institutional Animal Care and Use Committee of the Lovelace Institutes and East Carolina University School of Medicine. Adult Long-Evans hooded rats (males, 330 \pm 26 g; females 275 \pm 13 g; 60 to 70 days old) were used for all experiments.

Surgical Preparations. Neonate male pups were castrated within the first 5 days of birth to prevent testosterone production during maturity. During the brief surgical procedure all pups were anesthetized with 3% halothane and 97% oxygen using a small animal anesthesia machine (Summit Hills Laboratories). An incision was made in the scrotal area and both gonads were surgically removed. Dissolvable sutures were used to ligate vessels attached to the gonads to prevent any bleeding. After a 5 minute recovery period on a heating pad the pups were rejoined with their mother and reared to adulthood.

For the experiments on female sex hormones, two to four rats were randomly selected each day for implantation surgery. Prior to surgery, each animal was weighed and anesthetized by i.p. injection (90 mg ketamine and 10 mg xyalzine per ml; 0.1 ml/100 gm body weight.

Animals in two of the three groups were ovariectomized using a ventral abdominal incision. Animals in the third group were sham ovariectomized using a similar incision. Before closing the incision, animals from all groups also had Mini-mitters (Mini-Mitter Inc., Sun River, OR) implanted in their abdomens. These mini-mitters were calibrated prior to the surgery using the program DataQuest III and sterilized in a caustic compound. In animals from one ovariectomy group (OVX-E), had a pellet containing 0.5 mg 17-b estradiol in cholesterol implanted subcutaneously. This concentration is considered to provide a replacement dose of estradiol to the animals in this group. Pellet were designed to release over a three week period.

Following surgery, animals were returned to their cages and allowed to recover for six to nine days. Laboratory personnel performing the tolerance testing were blinded as to hypotheses and groups. In the laboratory, each rat was placed in a special chamber in which both air composition and temperature could be controlled. Each Plexiglas chamber had an inner dimension of 16 x 16 x 14.5 cm. Two of the chambers were kept at 30°C and the other two at 18°C.

Hypoxia tolerance. Each animal was exposed to acute graded hypoxia (21, 16, 12, 10, 8, 6, 4, and 2% inspired O₂) for 30 min at each level. A continuous supply of various O₂ and N₂ gas mixtures were flushed through the chamber at a rate of 1500 ml/min with a gas mixing flowmeter (Cameron Inst. Co.). Ambient temperature within the environmental chamber was regulated with an external heating/cooling water bath (Neslab RTE-110, Newington, New Hampshire). The tolerance level was defined as the concentration of inspired O₂ at which the rat exhibited loss of posture (ataxia). Immediately after the start of ataxia the rat was removed from the chamber. In almost all cases, the rats recovered with no apparent complications.

Ventilation Measurements. Ventilatory frequency (f), tidal volume (V_T), and steady state minute ventilation (\dot{V}_E) were measured in temperature clamped and hypothermic rats during exposure to inspired O_2 concentrations ranging from 24% to 8%. In Albuquerque (5200 ft; 1646 m) 24% inspired O_2 represented an equivalent O_2 concentration for sea level PO_2 and the lowest O_2 level, i.e. 8%, is equivalent to 25740 ft (7800 m). Ventilation measurements were obtained using a plethysmographic technique.

Oxygen Uptake Measurements. Oxygen consumption was assessed prior to ventilation measurements at the end of each 1-hour level. An O_2 analyzer (model S-3A; Electrochemistry, Sunnyvale, CA) measured O_2 concentration of the gas flowing into and out of the environmental chamber. Gas outflow was measured by volume displacement using an immersed graduated cylinder. The respiratory exchange ratio (R) was not measured, so values were not corrected for conditions of $R < 1.0$. However, with the flows employed in this study (700 ml / min), error analysis shows that ignoring $R \neq 1$ results in a trivial change in calculated $\dot{V}O_2$.

Statistical Analyses. Data were analyzed using a two-way analysis of variance (ANOVA) for repeated measures. Gender and T_b (i.e., male/female, castrated/non-castrated, estrus/mixed estrus, hypothermic/temperature clamped) comprised the between-subjects ANOVA factor. Level of inspired O_2 (%) comprised the within-subjects ANOVA factor. Simple main effects were analyzed to assign between group differences at specific levels of O_2 . All comparisons in gender studies were based on litter mates. Tukey's post-hoc tests were used to assign significant differences within the levels of inspired O_2 factor. In all cases, a $p < 0.05$ difference was considered significant. Values are presented as means \pm SD. Tolerance curves were compared using the log-rank test (Mantel-Haenszel test)

Results

Tolerance of extreme hypoxia and body temperature. Female rats were more tolerant of extreme hypoxia than male rats. This was the case when exposure took place in the 30°C chamber (normal body temperature) or 20°C chamber (hypothermic) (Fig. 1). In the warm chamber, there was no gender difference in T_b under normoxic or hypoxic conditions (Fig. 2). Male rats, but not females showed a slight, but significant, drop in T_b during extreme hypoxia (37.55 to 36.75°C) but were still less tolerant than females. In the room temperature chamber, there was a significant effect of both hypoxia and gender, with T_b dropping from 37.4 to 30.9°C in males and from 37.5 to 29.72°C in females.

Physiological basis for increased tolerance of female rats. In rats kept at normal T_b , the hypoxic ventilatory response was significantly higher for females (Fig. 3). The component of minute ventilation that accounts for the gender difference was an increase in tidal volume with no difference in frequency. In rats kept in a room temperature chamber so that hypothermia ensued ($T_b \approx 32^\circ C$) during extreme hypoxia, there was no gender difference in ventilatory response (Fig. 4) but there was a significant difference in T_b , as noted above.

Mechanism of the gender difference. A potential role was examined for both female and male sex steroids. The results of the ovariectomy experiments on hypoxia tolerance are shown in Figure 5. The increased tolerance of hypothermic female rats compared with females kept at normal T_b was abolished by ovariectomy. This was accompanied by a slight, but non-significant rise in T_b from 27.1 to 28.6°C.

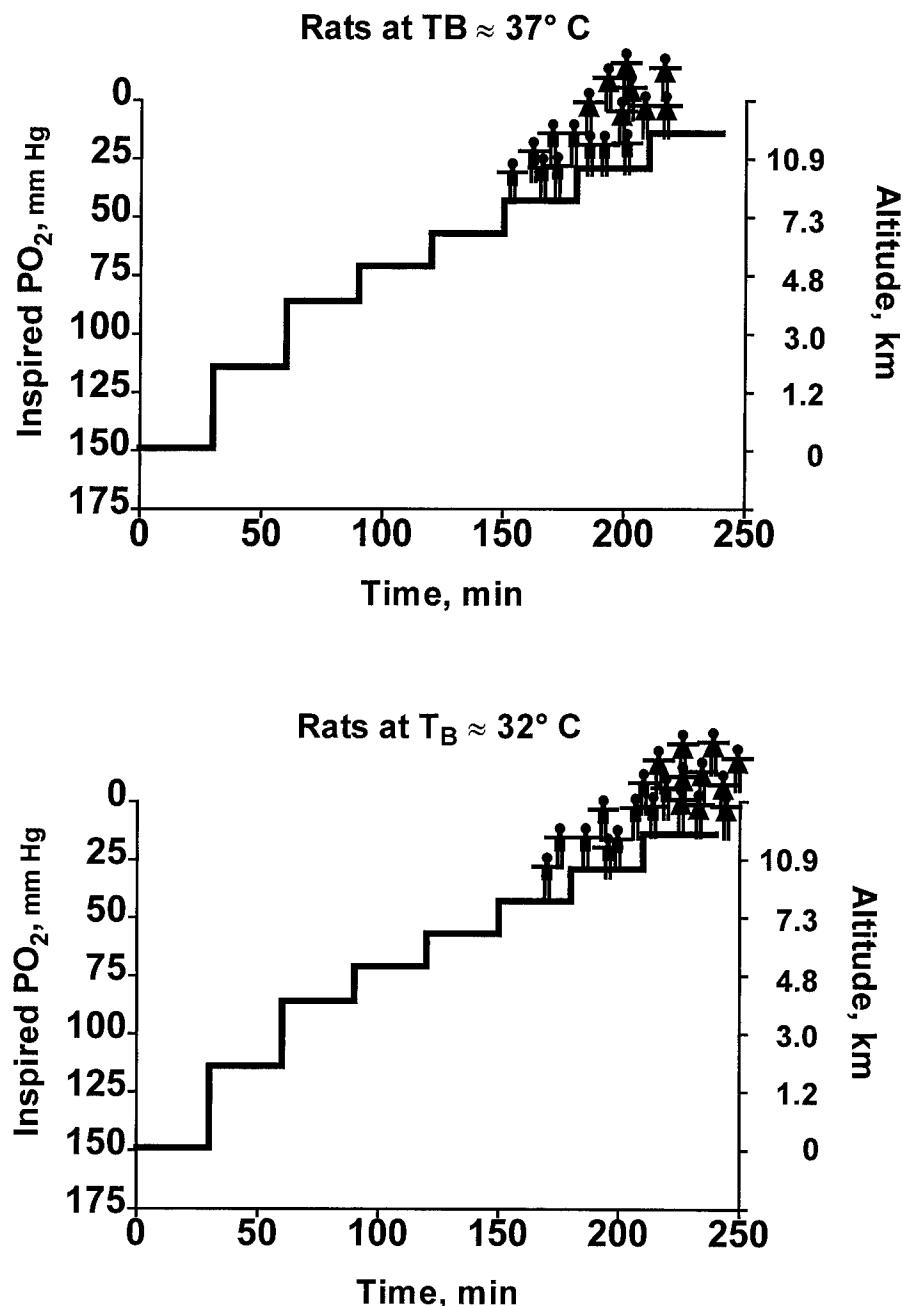


Figure 1 Altitude profile and hypoxia tolerance of male and female rats at normal body temperature (upper panel) and when hypothermic (lower panel).

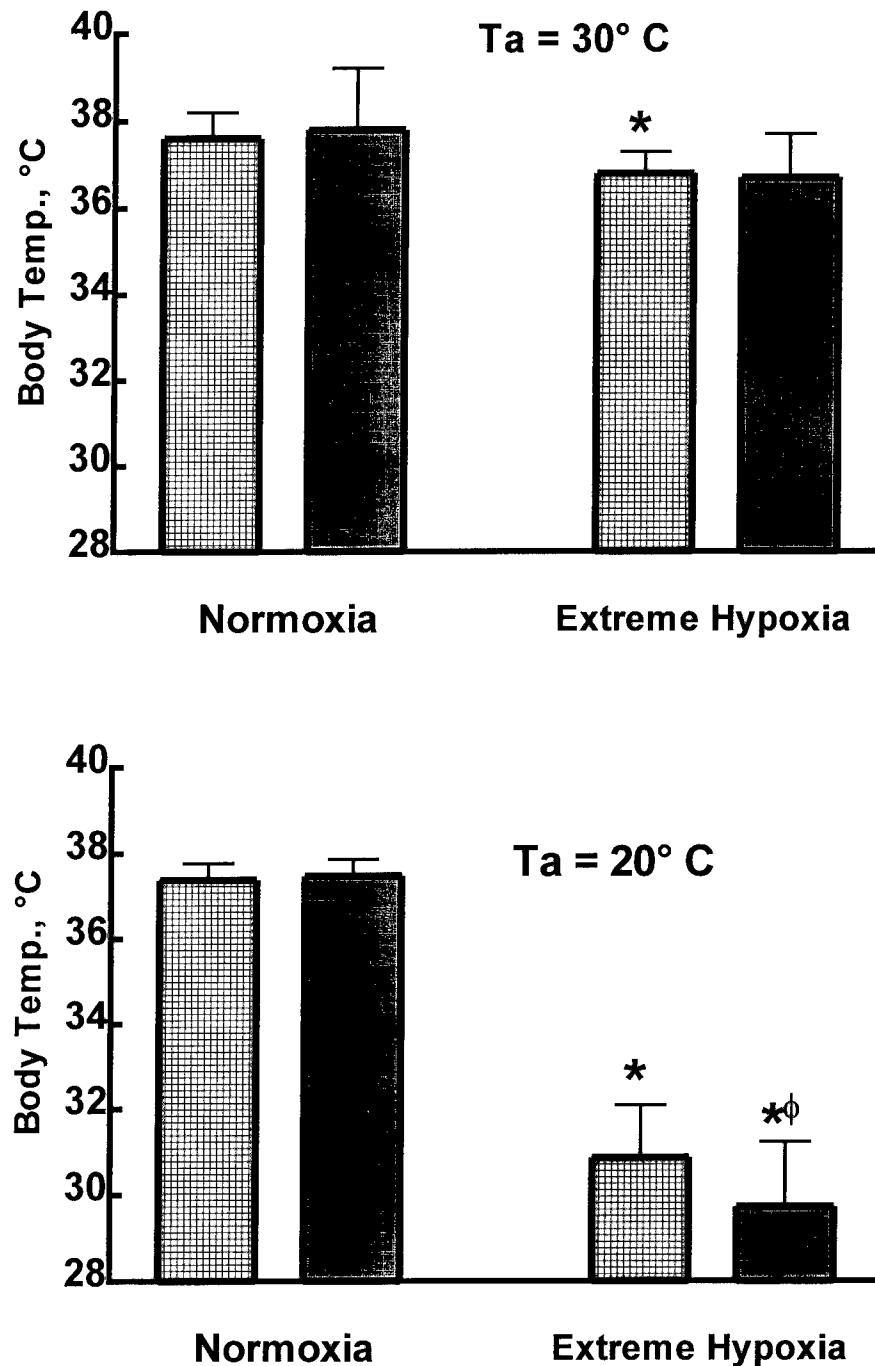


Figure 2 Body temperature of male (checkered bars) and female (solid bars) rats under normoxic and extreme hypoxic conditions when kept at ambient temperature of 30°C (upper panel) and 20°C (lower panel).

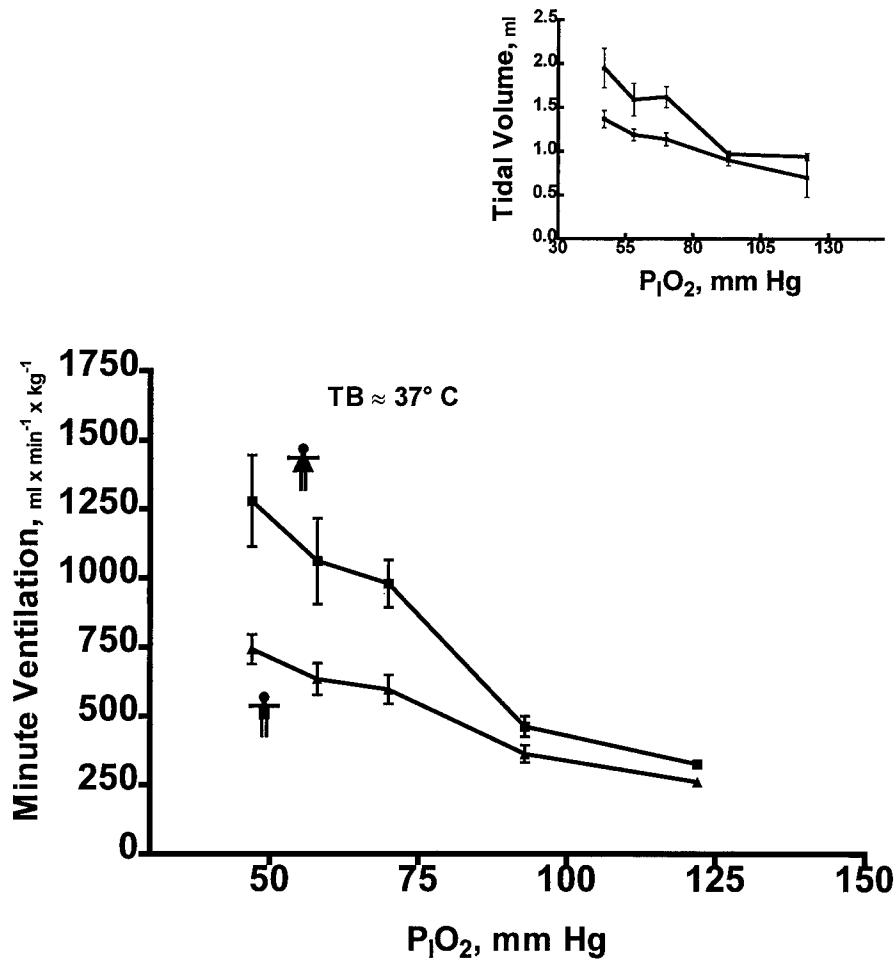


Figure 3 Minute ventilation of male and female rats kept at normal body temperature during graded hypoxia.

Estrogen replacement therapy had a marginally significant effect on restoring hypoxia tolerance, but came nowhere close to control values. In females kept at normal T_B , ovariectomy had no effect on hypoxia tolerance. In this group, there was a significant gender difference in hypoxia ventilatory response correlated with a gender difference in hypoxia tolerance. The difference in hypoxic ventilatory response when female rats at peak estrus (minimum progesterone) were compared with males (Fig. 6) was diminished but not abolished. However, when castrated males were compared with control males, an inhibiting effect of testosterone on HVR was revealed (Fig. 6). The gender difference could only be abolished by both progesterone reduction and testosterone reduction.

Discussion

Previous studies showed female dominance in hypoxia tolerance. Stupfel et al.¹² showed increased survival of female rats and mice during nitrogen breathing. In a

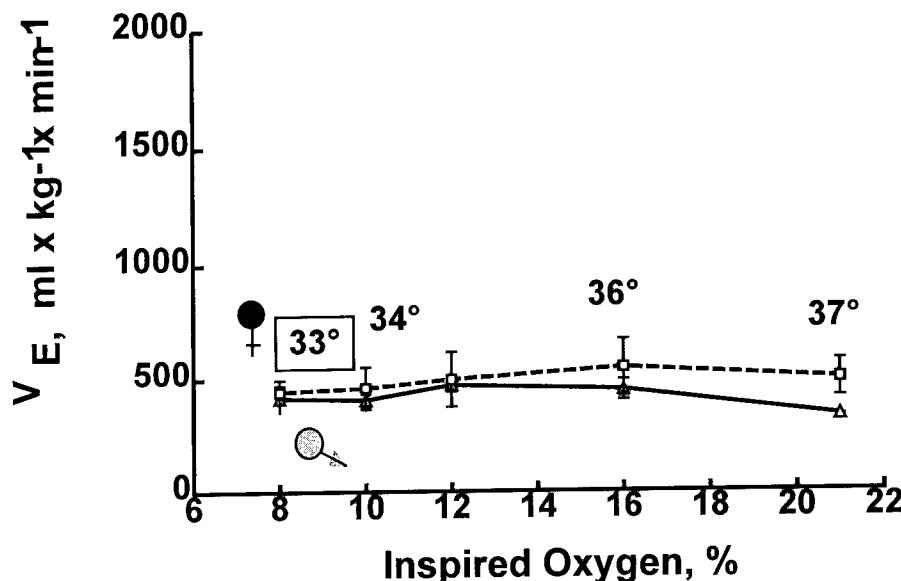


Figure 4 Minute ventilation of male and female rats when hypothermic during graded hypoxia.

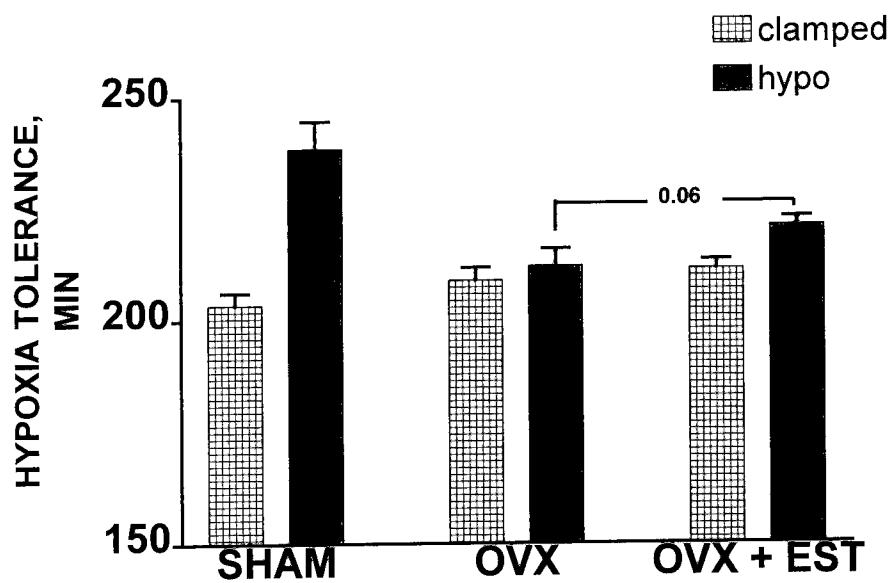


Figure 5 Hypoxia tolerance in female rats at normal body temperature (checked bars) and when hypothermic (solid bars) in sham operated, ovariectomized, and ovariectomized plus estrogen replacement groups.

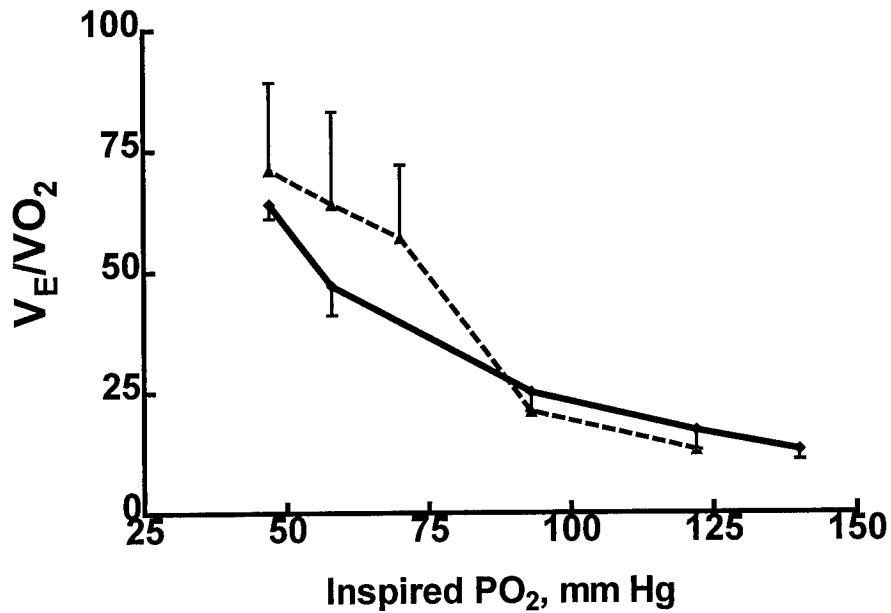


Figure 6 Minute ventilation, corrected for oxygen uptake, of female rats at normal body temperature when measured in mixed estrus (dashed line) and peak estrus (solid line) which corresponds to minimum progesterone levels.

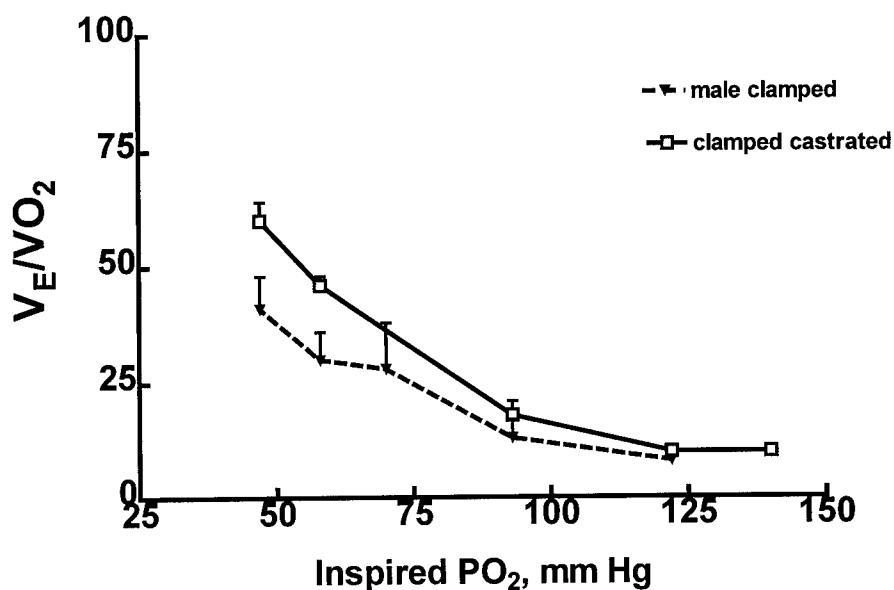


Figure 7 Minute ventilation, corrected for oxygen uptake, of male rats at normal body temperature comparing intact and castrated rats

strain of rats sensitive to mountain sickness, female rats developed a less severe polycythemia and tolerated prolonged hypoxia better than male rats.⁹ In a subsequent study, Ou et al.¹⁰ showed that the gender difference in rats was attributable to female sex hormones.

There appears to be a dual mechanism for increased tolerance of females to extreme hypoxia. When animals are maintained at normal T_B , the increased HVR of females may be the crucial difference. There was no difference in the oxygen consumption of clamped and hypothermic rats during hypoxia, so the increase in ventilation was an increase in the air convection requirement (V_E/VO_2). It is plausible that this increased ventilatory response confers greater survival potential in the female rats by increasing delivery of available oxygen during the hypoxic exposure. The results of the peak estrus (minimum progesterone) study are not surprising as there is ample evidence in other species of a stimulatory effect of progesterone on female ventilation. The inhibitory effect of testosterone on HVR was not expected, although there is evidence for rats that castration results in increased lung volume.⁸ The present results are also consistent with previous data showing that female rats have higher compliance and lower airway resistance than male rats.^{7,17}

Hypoxia in rats is also known to inhibit nonshivering thermogenesis.⁴ Shivering is also inhibited by hypoxia.⁵ These are the reasons that rats kept at room temperature, which is considerably below their thermal neutral zone, become hypothermic during hypoxia. In our study, male and female rats in the hypothermic group exhibited pronounced shivering after being taken out of the hypoxic chamber, but not during the exposure itself.

When animals are permitted to become hypothermic, there is no gender difference in HVR but there is a lower T_B in female rats. Even though the difference in T_B is only 1°C, this may be significant in preserving brain metabolic status, as shown in previous studies on rats.^{2,16} Berntman et al.¹ showed that a 1°C drop in brain temperature is adequate to prevent a fall in brain adenosine triphosphate levels at a Po_2 of 20 mm Hg.

One concern with this result was the significant size difference of male and female rats of the same age. Depending on the mechanisms of heat loss and heat gain, such large differences could directly affect changes in T_B . However, there was no statistically significant relationship between changes in T_B and body mass in this study.

It may be that the moderate hypothermia that accompanies extreme hypoxia extends female tolerance more than that of males due to a protective effect of estrogen and/or brain hypothermia on brain function. When hypothermia is prevented, the augmented ventilatory response of females may be the dominant factor in the gender difference.

Acknowledgements

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CHAPTER 15

AUTONOMIC CARDIAC REGULATION DURING ACUTE AND CHRONIC HYPOXIA ASSESSED BY SPECTRAL ANALYSIS OF R-R INTERVAL VARIABILITY

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Introduction

It has long been recognized that the R-R interval of the heart rate is not constant from beat-to-beat. In 1733, Stephen Hales observed the relationship between respiratory cycle, arterial pressure and interbeat interval.¹⁰ Only recently has it been observed that this short term variability in R-R interval is mediated by the sympathetic and parasympathetic nervous systems (SNS and PNS, respectively) and that by quantifying this variability one can assess the relative contributions of the two limbs of the autonomic nervous system to heart rate variability. The most important applications of spectral analysis of heart rate variability to date have been in fetal heart rate monitoring and in the assessment of patients with diabetes or post myocardial infarction. Specifically, low R-R interval variability is associated with fetal hypoxia and with an increased risk for sudden cardiac death.^{5,15} Spectral analysis of R-R interval variability allows an assessment of 1) the magnitude of R-R interval variability and 2) the frequencies at which the variability occurs.

The attractiveness of using spectral analysis to measure autonomic cardiac regulation lies in the non invasive nature of the method, the ease with which the measures can be performed, and the well-established reproducibility of the results.^{9,15}

Physiology of Autonomic Cardiac Regulation

Pacemaker cells exist in cardiac tissue and they confer an intrinsic automaticity to the heart. However, in the normally functioning heart, rate, rhythm, and contractility are largely controlled by the autonomic nervous system. Efferent SNS and PNS modulation of the heart occurs synchronously with each cardiac cycle.²⁰ Under resting conditions, the PNS is the predominant cardiac regulator and R-R interval variations are primarily vagally controlled. The effect of PNS activity on the heart is mediated by release of acetylcholine from the vagus nerve. This acetylcholine binds

to muscarinic (M2) receptors on pacemaker cells in the sinoatrial (SA) node, causing an increase in cell membrane potassium conductance^{26,32} and ultimately decreasing the rate of pacemaker cell firing.

Sympathetic regulation of the heart is mediated by release of norepinephrine from cardiac sympathetic nerves and epinephrine from the adrenal medulla. Catecholamines act on sinoatrial pacemaker cells via B-adrenergic receptors. Ligand binding causes G-protein mediated increase in intracellular cAMP levels, ultimately accelerating the slow diastolic depolarization and increasing heart rate.

2 Primary Rhythms Mediate Short Term R-R Interval Variability

Due to continuous alterations in the sympathovagal balance, there is considerable R-R interval variability with respect to the mean R-R interval. There are two primary physiologic rhythms that mediate this R-R interval variability and each is controlled by a different limb of the autonomic nervous system: 1) the PNS-mediated respiratory sinus arrhythmia and 2) the SNS-mediated baroreflex.

Respiratory Sinus Arrhythmia: The respiratory sinus arrhythmia mediates periodic changes in heart rate corresponding to the respiratory rate. Due to inspiratory inhibition of vagal tone, R-R interval decreases with inspiration and increases with expiration.⁷ The mechanism for this rhythmic heart rate variability is thought to have both a central and peripheral component. The dominant control of the respiratory sinus arrhythmia is a central communication between the medullary respiratory center and the cardiovascular center. Respiratory sinus arrhythmia is controlled to a lesser extent by stimulation of thoracic stretch receptors.¹ Several studies have demonstrated that the respiratory sinus arrhythmia disappears with either surgical vagotomy or muscarinic blockade using atropine,^{2,23} further indicating that the respiratory sinus arrhythmia is mediated by the PNS.

Baroreflex-linked R-R Interval Variability: The baroreflex mediates R-R interval variability with a periodicity of 10 seconds. This "10-second rhythm" originates from intrinsic oscillations in the vasomotor component of the baroreflex circuit and is caused by negative feedback in the baroreflex loop.²⁹ The oscillations in baroreceptor firing are paralleled by synchronous fluctuations in arterial pressure known as Mayer waves.²⁷ Studies in involving B-adrenergic blockade in humans and cardiac sympathectomy in dogs have demonstrated that this heart rate variability is controlled by the SNS.^{2,28}

Spectral Analysis of R-R Interval Variability

The first step in spectral analysis of R-R interval variability is the extraction of a series of consecutive R-R intervals from an electrocardiogram. From this, a fast fourier transform analysis is performed on the R-R interval variability to determine specific frequencies at which R-R interval varies. An advantage of spectral analysis over other methods used to analyze R-R interval variability is that it provides a measure of both the magnitude of heart rate variability and the amount of variability that occurs at defined frequencies.^{20,33} The total amount of variability over the frequency ranges of interest is called the power spectral density (PSD). Simply stated, spectral analysis involves breaking down the sequential R-R intervals into the sum of a series of sinusoidal functions of different frequencies and amplitudes using the Fourier transform algorithm.¹⁹ It should be noted that there are several methods for calculating the total amount of variability and that each of these methods

provides only an estimate of the true power spectral density.¹³ The two main classifications of methods of spectral analysis are parametric and nonparametric and it has been demonstrated that both methods provide similar results.¹⁹

To illustrate how spectral analysis converts R-R interval variability from time domain to frequency domain, let us consider the respiratory sinus arrhythmia. If breathing is controlled at 12 breaths per minute, the period of each breath and therefore the period of respiratory-linked R-R interval variability will be 5 seconds. Since frequency equals the inverse of period, the frequency that corresponds to the respiratory sinus arrhythmia is .2 cycles per second (Hz). On a plot of spectral density versus frequency, one would expect to observe a large amount of R-R interval variability at 0.2 Hz (Fig. 1).

Interpretation of Frequency Plots of R-R Interval Variability

Spectral analysis of R-R interval variability produces a plot of power spectral density (PSD) versus frequency. The frequency range of interest for assessing short term modulation of the heart is 0.04-0.40 Hertz. R-R interval variability that occurs from 0.15-.40 Hz is designated high frequency variability. Efferent vagal activity is responsible for the majority of the heart rate variability in this frequency range, as seen in experiments involving electrical vagal stimulation, vagotomy, and pharmacological blockade of muscarinic receptors.^{2,20,28} High frequency heart rate variability can be expressed in absolute values of variability or in normalized units, which represents the power in the high frequency range in proportion to the total power

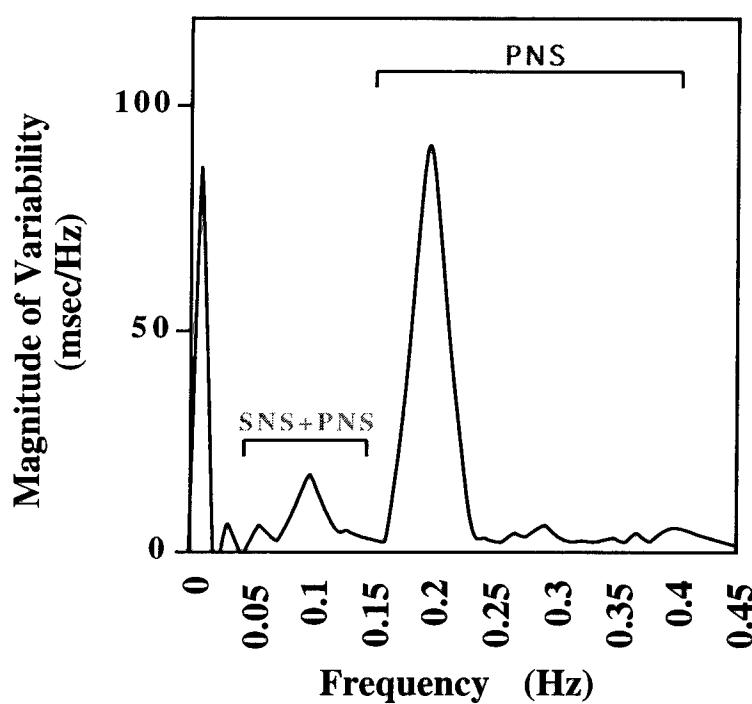


Figure 1 Frequency plot of R-R interval variability at altitude.

minus very low frequency variability (less than 0.04 Hz).¹⁹ Normalization of the units minimizes the effect of changes in total power on the value of the high frequency component.

R-R interval variability occurring in the range of 0.04-0.15Hz is thought to be mediated jointly by the sympathetic and parasympathetic nervous systems. During SNS activation, the amount of R-R interval variability in the low frequency range can increase or decrease, making the absolute power of low frequency variability difficult to interpret. This can be explained by recognizing that the PNS also contributes to LF variability and that SNS activation will be accompanied by decreased parasympathetic modulation of the heart, thus producing a reduction in total power of R-R interval variability.¹⁹ To circumvent this problem, the ratio of low frequency: high frequency R-R interval variability is used as the indicator of sympathetic modulation of heart.

The Effect of Acute and Chronic Hypoxia on Sympathoadrenal Activity As Assessed by Serum and Urinary Catecholamine Levels

Exposure to high altitude is a stressor that induces alterations in sympathoadrenal activity necessary to achieve physiologic adaptation to hypoxia. Sympathetic neural activity can be assessed by measurements of serum and urinary norepinephrine levels, while adrenal medullary activity can be assessed by serum epinephrine levels. It is important to recognize that serum and urinary catecholamine measurements assess total sympathetic neural and adrenal medullary activity, while spectral analysis of R-R interval variability measures the autonomic modulation of the heart. These are distinct entities that are governed by different regulatory mechanisms. However, it is useful to compare the changes in sympathoadrenal activity with acute and chronic hypoxia to those of autonomic cardiac modulation.

Evidence indicates that there is a dissociation of sympathetic neural activity and adrenal medullary activity with acute and chronic exposure to altitude (Fig. 2 and 3).^{12,21} In males acutely exposed to high altitude, resting plasma epinephrine levels have been reported to increase relative to sea level values.^{12,14,21,31} Using arterial measurements, Mazzeo et al demonstrated a significant rise in serum epinephrine levels within 4 hours of exposure to an altitude of 4300m.²² The response of norepinephrine to acute hypoxia is somewhat different. Most studies demonstrate that norepinephrine levels do not change significantly with acute exposure to altitude,^{4,21,31,36} though other measures of SNS activity, including release of norepinephrine from the resting leg, indicate increased SNS activity.²² This finding illustrates the important point that serum norepinephrine measurements cannot detect regional differences in sympathetic neural activity.

With chronic exposure to high altitude, resting arterial epinephrine values decline significantly relative to values observed with acute exposure to altitude. The response of the SNS as assessed by arterial norepinephrine measurements is distinct from the response of the adrenal medulla. Mazzeo et al²¹ observed that with chronic hypoxia (21 days at 4300m), resting arterial norepinephrine levels increased 85% relative to sea level values. Other investigators have reported similar findings.^{30,36} In studying norepinephrine turnover in the rat heart, Johnson et al¹² demonstrated an increase of 130% after 7-14 days of exposure to 10.5% oxygen, indicating increased sympathetic neural activity in the heart with chronic hypoxia. These findings might lead one to hypothesize that with chronic exposure to high altitude, there will be

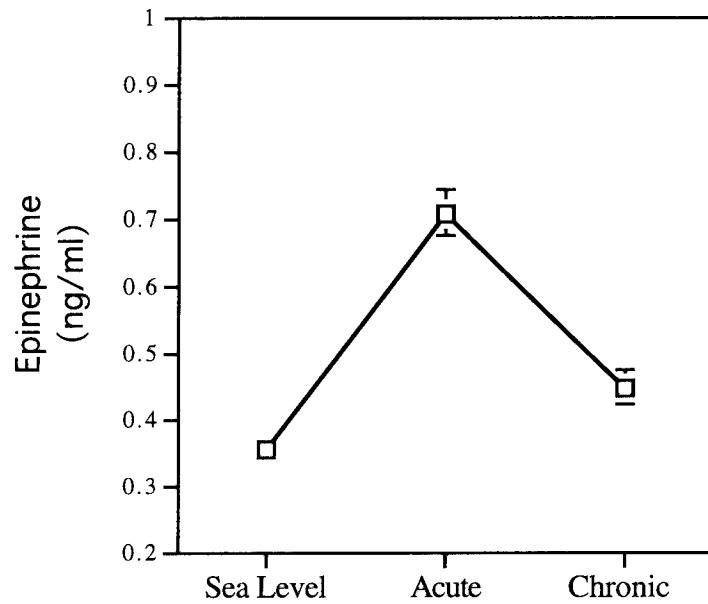


Figure 2 Resting arterial epinephrine determined at sea level as well as during acute and chronic high altitude exposure. Values are means +SE. Modified from Mazzeo et al.

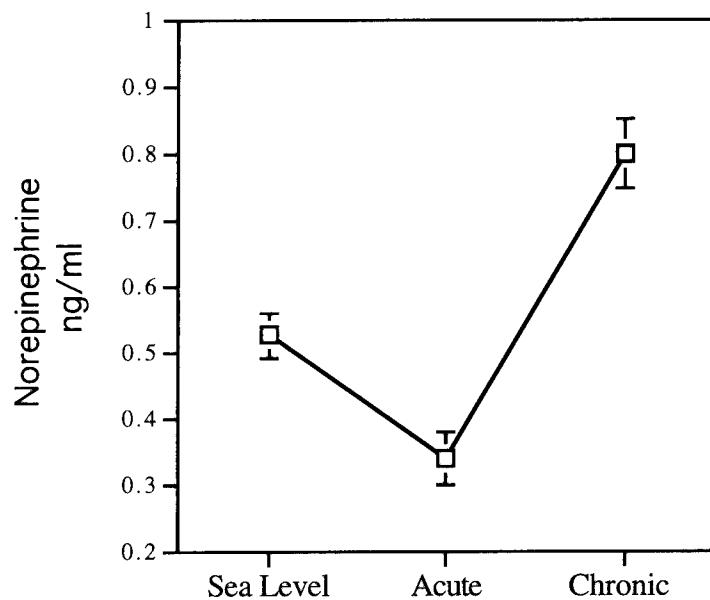


Figure 3 Resting arterial norepinephrine determined at sea level as well as during acute and chronic high altitude exposure. Values are means +SE. Modified from Mazzeo et al.

increased SNS cardiac modulation. In fact, this is not the case and it illustrates the point that serum norepinephrine assessment measures global sympathetic neural activity while spectral analysis of R-R interval variability measures only the effect of catecholamines on cardiac function.

The Effect of Acute and Chronic Hypoxia on Autonomic Cardiac Regulation

Spectral analysis of beat-to-beat heart rate variability can be used to assess the changes in short term autonomic cardiac regulation that occur with acute and chronic hypoxia. The role of SNS activation in adaptation to high altitude has been documented by elevations in serum norepinephrine with chronic hypoxia. However, the precise changes that occur in sympathetic cardiac modulation cannot be assessed using serum or urinary catecholamine levels. As mentioned previously, the ratio of low frequency: high frequency R-R interval variability provides an assessment of sympathovagal balance contributing to cardiac modulation.

The effect of altitude on PNS-mediated cardiac modulation has been less well established and has traditionally been difficult to assess non invasively. Spectral analysis of R-R interval variability provides a relatively quick and non invasive assessment of vagally-mediated cardiac modulation.

SNS Cardiac Modulation: Hughson et al¹¹ demonstrated that in men in a resting state, there is increased sympathetic cardiac regulation with 4-5 days of exposure to altitude compared to sea level values (Fig. 4). By days 11-12 at altitude, sympathetic cardiac modulation declined to values intermediate to those observed at sea level and altitude days 4-5. These findings indicate increased sympathetic cardiac

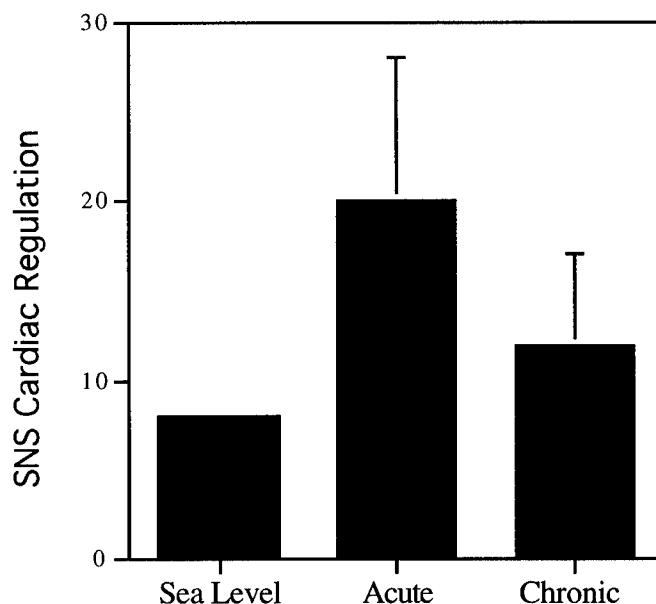


Figure 4 Mean values (+- SE) of sympathetic cardiac regulation (low frequency: high frequency variability) during supine rest at sea level, early altitude exposure (days 4-5), and chronic altitude exposure (days 11-12). Modified from Hughson et al.

modulation with acute hypoxia that declines with increasing duration of hypoxia. Other investigators have observed similar changes in sympathetic modulation of the heart with acute hypoxia.^{17,18} There is little other data assessing the effect of chronic hypoxia on sympathetic cardiac modulation.

PNS Cardiac Modulation: With acute exposure to altitude (day 4-5), Hughson et al¹¹ observed a decrease in PNS mediated cardiac regulation relative to sea level values, followed by an increase in parasympathetic cardiac modulation by days 11-12 at altitude (Fig. 5).

Hughson et al observed a significant increase in heart rate in men with acute altitude exposure followed by a decrease toward sea level values by days 11-12 at altitude. The results of spectral analysis of R-R interval variability suggest that the increase in heart rate observed with acute hypoxia is mediated by both increased sympathetic cardiac modulation and decreased parasympathetic cardiac modulation.¹¹ However, the relative importance of changes in SNS and PNS modulation of heart rate is unknown. With chronic exposure to altitude, a trend toward decreased SNS cardiac modulation and increased PNS cardiac modulation was observed and may contribute the decreased resting heart rate observed relative to sea level values.¹¹

It is interesting to note that there is decreased sympathetic cardiac modulation with chronic hypoxia despite elevated urinary and serum norepinephrine levels. A possible explanation of this finding is downregulation of cardiac beta-adrenergic receptors. Studies in rats have demonstrated a decreased number of receptor sites after 5 weeks of chronic hypoxia,³⁵ while studies in humans demonstrate a decreased

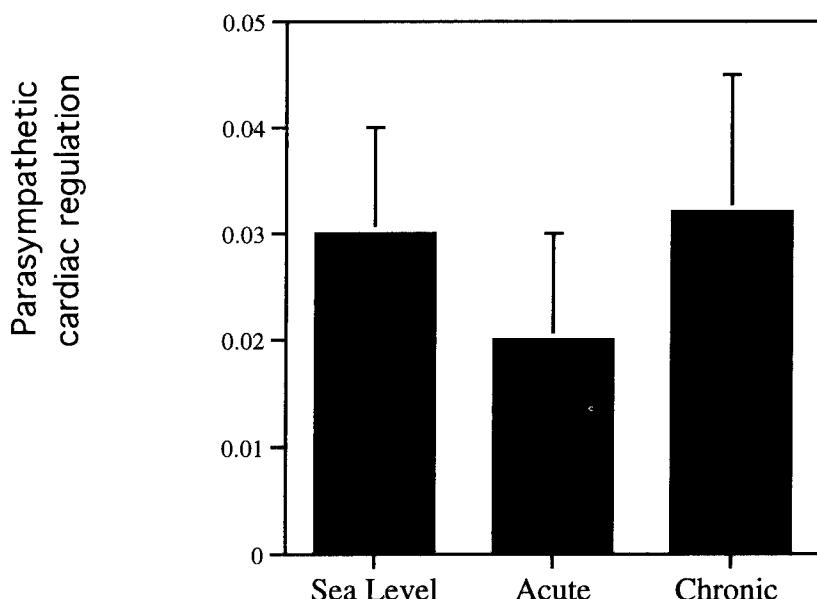


Figure 5 Mean values (+- SE) of parasympathetic cardiac regulation (high frequency variability/total power) during supine rest at sea level, early altitude exposure (days 4-5), and chronic altitude exposure (days 11-12). Modified from Hughson et al.

response to isoproterenol after prolonged exposure to high altitude.³⁰ A current hypotheses explaining this finding is that sustained SNS activation causes a decrease in the number of cardiac B-adrenergic receptors, thereby decreasing cardiac tissue response to SNS stimulation.^{11,30,35}

Hypotheses Concerning the Effect of Ovarian Hormones on Sympathoadrenal Activity

Ovarian hormones interact with sympathoadrenal mechanisms of vascular control in a number of ways. Vasodilatory actions of estradiol are chiefly mediated by activation of endothelial alpha₂-receptors, which increase endothelial production of nitric oxide and vasodilator prostaglandins.^{25,34} Estradiol also has alpha₁-adrennergically mediated vasoconstrictor effects; estradiol increases the number and affinity of alpha₁-adrenergic receptors to raise contractile response to norepinephrine.^{3,6} Progesterone is also known to relax venous smooth muscle,²⁴ but generally opposes alpha₂-mediated vasorelaxation.²⁵ The combined actions of estradiol and progesterone on sympathoadrenal mechanisms are complex and the effect on autonomic cardiac regulation has not been demonstrated. Studies are currently underway to determine the effect of altitude and menstrual cycle phase on autonomic cardiac modulation.

Conclusion

Though it has been demonstrated that alterations in sympathoadrenal activity contribute to acute and chronic adaptations to hypoxia, the changes in SNS- and PNS-mediated cardiac regulation that occur with hypoxia are not well understood. Spectral analysis of R-R interval variability is a simple, non invasive technique that allows for a quantitative assessment of sympathetic and parasympathetic regulation of the heart. Further studies are needed to assess: 1. the effect of changes in adrenergic receptor number and function on cardiac regulation and 2. the effect of ovarian hormones on adaptation to acute and chronic hypoxia in women.

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CHAPTER 16

CRAGS AND CRINOLINES

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Wichita
Kansas

"A widely spread interest in Alpine travel has thus grown up in our family circles, making wives and sisters seek participation in the pleasures which they hear so vividly described....."

Mrs. Henry Freshfield, 1861

The European woman of the Victorian era lived in a man-dominated society where she was trained to live an ideal life of womanly submission and strict self-discipline. Well defined classes of society with rigorous social rules and religious devotion kept many women house-bound. They were fettered with social obligations, tightly bound clothing, and a multitude of servants, despite resources of money and education. From this setting sprang a surprising number of women who chose to travel out of the house and into the mountains: Henriette d'Angeville, Lucy Walker, Meta Brevoort, Elizabeth LeBlond, and Fanny Bullock Workman, are the few mentioned here. They achieved great heights and daring feats despite rebuke from their women friends, doubt from alpine guides, disdain from men, as well as the expected weather and terrain obstacles. The 19th century woman climber tried to balance the Victorian expectations of feminine dress and decorum with the rigorous demands of rock and ice, often climbing rugged crags with bonnet, scarf, pockets laden with personal necessities, long skirts and crinoline.

Henriette d'Angeville (1794-1871)

Born during the Reign of Terror and a member of the French aristocracy, Henriette's family moved to the Jura area after her grandfather was guillotined and her father imprisoned. As a teenager she scrabbled about the small limestone hills, but it was not until she was 44 years old that she made her first climb. There are many theories as to why she had so great a passion to climb Mont Blanc, even that she was 'a spinster who loved Mont Blanc because she had nothing else to love.' Whatever her reasons, her highly publicized climb earned her the title of Bride of Mont Blanc.¹

Henriette was not the first woman to the top of Mont Blanc. Maria Paradis, a servant girl and native of Chamonix, was convinced to go to the top of Mont Blanc in 1809 by guides who dragged, pushed and carried her. This adventure brought Maria fame and profit, for many people stopped at her small tea shop to hear her story in the following years. However, she did not endeavor to climb any more mountains, and so faded from history.^{1,7,11}

Henriette's climb was preceded by much organization and planning on her part, and debate and discouragement from friends and the public. She had only 5 supporters for her mission. In Chamonix, many thought her mad. There were odds of 1000 francs to 5 that she would not reach the top.⁷ Although she was slow, feverish, weary and staggering during the climb, she revived immediately upon reaching the summit.^{7,11} The fame and notoriety of her success followed her for many years.

Mont Blanc was only the beginning for her, she continued to climb for another 25 years, making at least 21 more ascents, including a winter climb. In 1863, at the age of 69, she climbed the Oldenhorn, with the comment, "the Oldenhorn is my twenty-first alpine ascent, and will probably be one of the last; for it is wise at my age to drop the alpenstock before the alpenstock drops me."¹¹ She died in Lausanne in January 1871, just months before the Matterhorn was climbed for the first time by a woman, an event she most certainly would have cheered.

Lucy Walker (1835-1916)

One of the first Victorian women to climb regularly, Lucy began climbing at the age of 22 with her father and brother. The family went every year to the Alps, moving from one mountain district to the next.

Lucy made several "first lady's ascents" of peaks.¹ In 1862 she did the Dufourspitze of Monte Rosa (15,002 feet/4557.6 meters) with her father and lifetime guide, Melchior Anderegg. She was the first woman to climb the Weisshorn and the Lyskamm, and in 1864 with her family made the pioneer ascent (first for man or woman) of the Balmhorn. Her quiet nature and unassuming manner won her great admirers in mountaineering and social circles. In Whymper's famous picture *The Clubroom at Zermatt*, she is the only woman in the group. She never wore breeches, but always climbed in voluminous skirts, and always maintained the feminine role of climbing with her father and brother as chaperones. She never married. Once when asked why, she replied "I love mountains and Melchior, and Melchior already has a wife."¹¹

On doctor's orders in 1879, she gave up long expeditions, but continued to visit the Alps and her large circle of friends in the Oberland. Her popularity grew, and she was a much sought after speaker at dinners and gatherings. She was one of the first members of the Ladies' Alpine Club when it was founded in 1907, and in 1912 became the second president of the organization at the age of 76. She died peacefully in Liverpool.

Meta Brevoort (????-1876) and Tschingel (1865-1879)

Meta came to Europe in September 1865, just 2 months after the Whymper party's disaster on the Matterhorn. She was accompanied by her 15 year-old nephew—a fat, chronically ill boy. Her hopes that the climate of the Alps would improve his health became true. They soon became an accomplished climbing team, with the nephew eventually gaining fame and controversy as a mountaineer and historian.

Meta was also one of the first women to climb regularly in the Alps. She had a vivacious and colorful personality, and an immense capacity for enjoying everything. When she climbed Mont Blanc, on the summit she danced a quadrille in the snow and sang a spirited rendition of the *Marseillaise*. She was a heavy-set woman determined to climb in flannelette drawers, boned corsets, calico chemises and

skirts. She constantly experimented with her outfits, never being quite satisfied with their functionality.¹

In 1868, on the occasion of their party's failure to climb the Eiger due to poor conditions, an Oberland guide gave Meta's nephew a dog in consolation. This brown and white beagle bitch, named Tschingel after the first pass she crossed as a puppy, climbed with them for 9 years, making ascents of 66 major peaks and 100 minor expeditions. She once rediscovered the route when the climbing party was lost, and became famous in her own right. After old age and blindness forced her retirement from climbing, Tschingel lived comfortably in Surrey until she died in her sleep in front of the kitchen fire.¹¹

Meta had an impressive record of winter ascents, most notably the Wetterhorn on the 15th January 1874, followed by the Jungfrau on the 22nd January 1874. Her greatest two ambitions were to be the first woman to summit both the Matterhorn, and the Meije in the Dauphiné. She successfully climbed the Matterhorn in 1871—disappointedly only a day or two after Lucy Walker made the top. Her second ambition was never realized, as she died suddenly from complications of rheumatic fever.

Elizabeth Le Blond née Hawkins-Whitshed (1861-1934)

Lizzie was British but grew up mainly in Ireland. She initially married Fred Burnaby, a soldier much older than she. In 1882, after her husband was killed in war in Egypt, Lizzie returned to the Alps, climbing Mont Blanc and the Grandes Jorasses. She also took up the newly popular sport of bicycling, making the trip from Switzerland to Italy by bicycle. It is said 'she trundled her machine over the mountain passes and when the descent became too much for the primitive brakes she attached a small tree by a length of rope and trailed it behind her, so maintaining her equilibrium.'¹¹

She remarried, and travelled in China, but was soon again widowed. It was after her third marriage, to Mr. Aubrey LeBlond, that she gained fame as a mountaineer. She made many winter and spring ascents, including Piz Sella, Piz Zugö and one of the longest winter expeditions, the Disgrazia. She was the first woman to lead guideless climbs, and in 1900 with Lady Evelyn McDonnel led the very first 'women's rope' up Piz Palü.¹¹

Lizzie was 'the personification of elegance' so wore climbing breeches discreetly hidden under a skirt until the last village had been passed and no people were in sight. Once, during a climb of the Rothorn, her skirt, normally carried in the guide's rucksack, was inadvertently left on the summit. The party had to re-ascend, rescue the skirt, and return late to Zermatt.¹¹

In 1895 Roman Imboden, son of her guide Joseph, was killed on the Lyskamm, and Lizzie lost interest in climbing the Alps. After that, she and Joseph Imboden climbed mainly in Norway, but her enthusiasm waned, and her climbing career soon came quietly to an end. She did not give up mountains, however. She was the primary force in founding the Ladies' Alpine Club in 1907 and was the first president. The organization brought credence and movement to women's mountaineering, and Lizzie was re-elected for a second term in 1932. She died while in office in 1934.

Fanny Bullock Workman (1859-1925)

Born in Worcester, Massachusetts, the daughter of a wealthy former governor, Fanny's early education took her to Europe where she studied in Paris and Dresden.

She married Dr. William Hunter Workman in 1881, then moved with him to Europe in 1889 'for the sake of his health.'¹⁰

His health must have dramatically improved, as the two made extensive and rigorous cycling tours from 1895-1899. They bicycled through Algeria, India, Europe, Ceylon and Java before becoming mountaineers. On their first Himalayan expedition from Kashmir to Ladakh, Fanny was 40 years old and William 52 years old. Known always as Fanny Bullock Workman, and never as Mrs. Workman, Fanny established an altitude record for women in 1906, with the ascent of Pinnacle Peak (22,810 feet/6930 meters) in the Nun Kun Range of India.¹⁰

Fanny was the driving force for the expeditions made by the duo, including explorations of the Chogo Lungma, Siachen and Hispar glaciers. Fanny was an admitted feminist, often smoked, nearly always bicycled, and believed in women's suffrage. One picture of her on a summit shows her displaying a 'Votes for Women' poster. The Workmans were prolific writers, publishing over 11 travel and mountaineering books, noted for dry clinical observation and magnificent photographs. She was only the second woman to address the Royal Geographical Society in 1905, with her report on the 'First exploration of the Hoh Lungma and Sosbon Glaciers'.¹⁰

War broke out in 1914 after the Workmans' greatly successful exploration of the Siachen Glacier in 1911, and their travels were curtailed. In 1917 Fanny fell ill, and died in Cannes, France after 8 years of suffering. William returned to Massachusetts and survived her for another 13 years.

Readings

"Piz Scerscen Twice in Four Days" from Mrs. Aubrey Le Blond, *True Tales of Mountain Adventure for Non-climbers Young and Old* (1902)

"An Avalanche on the Schallihorn" from Mrs. Aubrey Le Blond, *True Tales of Mountain Adventure for Non-climbers Young and Old* (1902)

"A Day and a Night on the Bietschhorn" from Marguerite "Meta" Brevoort [credited to her nephew W.A.B. Coolidge], *Alpine Journal* 6 (1872)

Editor's Note: Doctor Sheri King's paper describing the Victorian lady mountaineers is a pale shadow of the brilliant monologue she delivered on Wednesday evening at the Symposium. In her gorgeous period dress with her climbing garb nearby, Sheri gave such a delightful performance that no one realized—or can believe—that she is not a professional and in fact had never done anything of the sort before. We are very grateful to her for this splendid act—and anticipate that she will be asked for many encores.

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CHAPTER 17

MABEL PUREFOY

FITZGERALD: HER LEGEND AND LEGACY IN HIGH ALTITUDE PHYSIOLOGY

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Introduction

Mabel Purefoy Fitzgerald was born in Preston Candover, England, in 1872 into a family in which the men were either in the clergy or the military, and the women raised the children. However, Mabel and her four sisters broke family tradition and did not marry and Mabel herself boldly went on to carve out her own career in medical science. It is at this point that a fascinating account of an intrepid woman, physiologist and scientist begins to unfold.

Some Background

When Mabel was in her early twenties, her life course shifted quite dramatically with the death of both of her parents in 1895: she and her sisters moved into a house at 12 Crick Road in Oxford and, leaving behind the rather free and easy lifestyle she had been leading up until then, Mabel began to pursue in earnest her career in laboratory medical science. Her interest in science had been sparked by her older brother Henry, a College Science and Chemistry teacher, and she had already taken some courses in Physiology in Shalstone where her grandmother lived, spending hours reading Huxley in the family garden. When she moved to Oxford in 1896, she continued with courses in the biological sciences and, interestingly, it was her top marks in these courses that moved Oxford to reverse its policy of not granting university degrees to women. Nevertheless, Mabel herself never did receive an official degree from Oxford until 1972 when she graciously accepted an Honorary MA on the occasion of her 100th birthday.

Research, Education and Career Profile

Mabel Fitzgerald's earliest exposure to clinical medicine was in Shalstone with a local doctor, Dr. De'Ath, during the time she was taking her first physiology courses: De'Ath introduced Mabel to the world of bacteriology and infectious diseases and from then on, her *curriculum vitae* becomes increasingly colourful and varied as she embarked on her career in laboratory medical science.

In 1898, Mabel who was now 26 years old, worked with Gustav Mann in Histology, studying in the areas of grey and white matter in different segmental levels of the spinal cord, work which was subsequently published under her name alone.¹ In 1900, she went to Copenhagen to work with George Dryer in Salomonsen's laboratory of Pathology, followed by a foray in Bacteriology at Cambridge, from which she published her second paper on the value of the opsonic index.² Her base throughout this period remained in Oxford, and it was at this time that she met both Sir William Osler and J.S. Haldane. Haldane was her neighbour on Crick Road and it was thus that he came to invite her to spend some time in his laboratory. Together they published a landmark study on the normal alveolar gas composition of men, women and children.³ Osler, on the other hand, played a key role in feeding Mabel's interest in clinical medicine by providing her with the opportunity to work with patients in the hospital. One of her studies included measurements of alveolar pCO₂ in patients with anemia, her interest in this area having been fuelled by the fact that her older sister Geraldine had died of iron deficiency anemia in 1900. Osler also got Mabel interested in old medical books and, on a number of occasions, encouraged her to get her medical training, a course which Mabel resisted for years. Interestingly, it was only when she reached her early forties that she considered this option seriously, but then was sidetracked once again and accepted a position as a Clinical Pathologist in Bacteriology and Lecturer in Edinburgh, Scotland, in 1915. Finally, Osler helped Mabel obtain a Rockefeller Institute Travelling Fellowship in 1908, by writing what must have been an excellent letter of reference. She moved to New York where she continued her work in Bacteriology, including a trip to Toronto, Ontario, to complete a study of gastric acid secreting cells with Professor McAllum, work which was also published solely under her name.⁴ And it was during this period in New York that Mabel's life and career began to gravitate towards mountains and high altitude physiology, although she kept up her work in Bacteriology and was sole author on yet another publication in this area.⁵

Colorado and High Altitude Physiology

During her stay at the Rockefeller Institute, Mabel visited the Rocky Mountains of Colorado twice, including Pike's Peak, which helped to entrench her love of mountains. In 1911, Haldane organized the first Anglo-American Expedition to Pike's Peak and asked Mabel if she would be involved: one would have to presume that her expertise with alveolar gas composition measurements coupled with her experience in New York and Colorado would be invaluable in terms of many of the logistical and scientific aspects of the expedition. Nevertheless it would have been "socially unacceptable" for a woman to spend 5 weeks alone on a mountain top with four men, *i.e.* Haldane and his colleagues, so instead she decided to travel through Colorado unaccompanied for the entire period! Indeed, using whatever local means of transport she could including horseback, Mabel shouldered her own Haldane apparatus and visited communities at 14 different altitudes from 5000 ft (1500 m) to 14,100 ft (4230 m) throughout Colorado, in order to make her alveolar pCO₂ measurements. She visited mining towns, smaller communities, the cities of Denver and Colorado Springs and the summit of Pike's Peak where Haldane and the men were doing their own experiments. She was able to demonstrate the inverse relation between atmospheric pressure and both alveolar pO₂ and pCO₂ and hemoglobin, and documented the clear gender difference in these measurements, *i.e.* women have a lower pCO₂ and hemoglobin concentration than men for each equivalent altitude.

These findings were published under her name only in what remains one of the cornerstone publications in high altitude physiology.⁶ The following year, Mabel collected similar data at the intermediate altitudes up to 4000 ft (1200 m) in the Appalachian mountains of North Carolina, findings which were also published with Mabel as sole author.⁷

Physiological Societies and Mabel Purefoy FitzGerald

In 1913, Mabel Purefoy FitzGerald was the second woman ever to be elected to the membership of the American Physiological Society (APS), based on her important contributions to science and physiology. Interestingly, the British Physiological Society waited until 1972 when Mabel turned 100 years old, to elect her to their membership, at the same time as Oxford, her old "alma mater", granted her an Honorary MA. Haldane in fact had been a key figure in first proposing in 1913, *i.e.* the same year that Mabel became a member of the APS, that women should be eligible for membership of the British Physiological Society,⁸ but this only came into effect two years later in 1915.

Conclusion

The contributions of Mabel Purefoy FitzGerald to our understanding of alveolar gas composition at altitude represent a cornerstone of our knowledge in high altitude physiology. Her major findings include documentation on normal alveolar gas composition in men, women, boys and girls, and the observation that the ventilation-dependent alveolar pCO₂ and the hemoglobin concentration in the blood decrease linearly with increasing altitude, with values in women always lower than those in men but following the same relationship to inspired pO₂.

Mabel was an intrepid woman, ahead of her time in the world of science which, at the time, was largely dominated by men. It is largely to her credit that not only were women allowed into the distinguished membership of the British Physiological Society in 1915, but also that Oxford began granting degrees to women. Other accomplishments include being sole author on a number of publications, which was very unusual in that period, and she became the second woman to become a member of the American Physiological Society.

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CHAPTER 18

MECHANISMS OF VENTILATORY ACCLIMATIZATION TO HYPOXIA *IN VIVO*

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The mechanisms responsible for the time-dependent increase in breathing on exposure to sustained hypoxia, termed ventilatory acclimatization to hypoxia (VAH), have remained elusive. Historically cerebral fluid acid-base changes surrounding central chemoreceptors were the major focus of interest²⁸. Recently there has been more emphasis placed on the peripheral arterial chemoreceptors as evidence for central chemoreceptor involvement waned¹⁰, and data accumulated that indicated that the carotid body (CB) response to hypoxia was increased after sustained hypoxia in animals^{2,11,31}. This was supported by the fact that the ventilatory response to acute hypoxia was increased in humans^{27,33} and animals^{1,12,31} during and after exposure to prolonged hypoxia. This paper will briefly review the basis for the goat model of VAH and describe mechanisms that have been explored using this model.

The importance of peripheral chemoreceptors in VAH was shown by studies indicating the significant attenuation of VAH after CB denervation in goats^{14,29}. These studies also showed that the time course of VAH is very short in goats, approximately four to six hours as compared to humans which require about ten days to acclimatize to an altitude of 4,300 m¹⁵. The awake goat isolated CB perfusion model was devised and with this model it was shown that the goat exhibits VAH with isolated CB hypoxia^{3,4}. These studies also indicated that brain hypoxia and CNS acid-base changes were not required to produce VAH in goats. Hypoxic CB simulation was unique in producing VAH as isolated hypercapnic CB stimulation failed to elicit VAH³. The findings in awake goats were supported by carotid sinus nerve studies showing time-dependent increase in CB afferent discharge frequency with steady-state hypoxia²², but not hypercapnia¹³. Recently, using the awake goat CB perfusion model it was shown that CB hypocapnia did not prevent the increased CB response to hypoxia that occurs during the prolonged exposure of the CB to hypoxia¹¹. This study also confirmed that the increased ventilatory response to hypoxia that persists for at least two hours of normoxia after VAH is due to elevated CB sensitivity to hypoxia.

A less complicated awake goat model of VAH has been developed¹². This model is the awake goat with one denervated CB and with both carotid arteries translocated to a subcutaneous position allowing intraarterial injections of pharmacologic agents on the side with the single intact CB or on the CB denervated side as control. The single CB is adequate for normal ventilatory function and is a feature of all of the VAH studies we have carried out including the CB perfusion experiments⁸. The goat is exposed to four hours of steady-state isocapnic hypoxia (Pao₂ 40 Torr) and exhibits a ventilatory response that is identical to four hours of isolated CB hypoxia⁵. This model of VAH exhibits the persisting acute increased ventilatory response to isocapnic hypoxia after return to normoxia that was demonstrated in CB perfusion studies. Isocapnic hypoxic exposure is used in this model because it minimizes cerebral acid-base changes which makes interpretation of data easier. Goats subjected to four hours of isocapnic hypoxia do not exhibit hyperventilation upon return to normoxia whereas those exposed to poikilocapnic hypoxia (allowing Paco₂ to fall) do have persisting hyperventilation after 4 hours of hypoxia¹².

Using this model potential CB noradrenergic mechanisms of VAH have been explored. It was found that ventilatory sensitivity to intracarotid infusions of norepinephrine were unchanged during and after VAH in goats²⁵. Similarly, sympathetic denervation of the CB did not change the magnitude and time-course of VAH in goats²⁶. These results suggest that CB-related noradrenergic/sympathetic mechanisms are not likely involved in VAH. These findings are compatible with those of Moore et al., who found no change in time course or magnitude of VAH in human subjects who were subjected to beta-adrenergic blockade²¹.

Recently the potential role of CB dopamine in VAH has been explored in the goat model. Dopamine is abundant in the CB and is known to be released during hypoxia¹⁷. Dopamine has been found to be inhibitory to the CB and results in reduced ventilation when given exogenously to animals and humans by intravascular injection^{6,19,32}. Furthermore, blocking CB dopamine D₂ receptors with a specific peripherally acting antagonist, domperidone, prevents this inhibition and increases the CB response and the whole animal ventilatory response to hypoxia^{18,30}. This formed the basis for the hypothesis that down-regulation of inhibitory CB dopaminergic mechanisms is responsible for increased CB sensitivity to hypoxia during VAH. Tatsumi et al. found support for this hypothesis in studies in which cats were found to have maximal ventilatory and CB responses to hypoxia after VAH which could not be further increased by dopamine receptor blockade with domperidone³⁰.

Further testing of this hypothesis has been carried out using the awake goat model. Three approaches have been used based on the assumption that dopamine is an inhibitory modulator of CB function: 1) If dopamine receptors are down-regulated during VAH, this should be detectable by ventilatory responses to intracarotid infusions of dopamine. 2) If there is a reduced release of dopamine from the CB or depletion of CB dopamine during VAH, causing increased CB sensitivity to hypoxia, then replacement of dopamine by intracarotid infusion would prevent increased CB sensitivity to hypoxia. 3) if dopaminergic inhibition is eliminated at maximal ventilatory drive during VAH, then blocking inhibitory dopamine receptors with domperidone should not further increase ventilation.

The first two of the above approaches have been undertaken and completed. In the first, dopamine has been given to goats via the carotid artery at the rate of 0.1, 1.0 and 10.0 $\mu\text{g}\cdot\text{kg}^{-1}$. This inhibits ventilation in a dose-dependent manner. The

response to dopamine was unchanged after VAH suggesting that dopamine receptor sensitivity was not altered during VAH.

In the second study, the goal was to provide an excess of dopamine to the CB which would prevent any reduction of dopamine mediated CB inhibition and thus prevent VAH. Dopamine was infused via the carotid artery to the intact CB at a dose of $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ throughout the four hour exposure to hypoxia. This dose will silence the CB during normoxia and attenuate the response to acute hypoxia. It was found that this regimen did not prevent VAH and did not prevent the increased ventilatory response to hypoxia following VAH. Thus, the mechanism responsible for increased CB hypoxic sensitivity during VAH in goats can overcome the effects of dopamine infusion; and furthermore, it is unlikely that VAH is related to a deficiency of dopamine at the CB.

In the third of these studies domperidone has been given intravenously after VAH is complete in the fourth hour of hypoxia. Preliminary results indicate that the response to domperidone remains robust (a further increase in ventilation) suggesting that the goat retains inhibitory dopaminergic function after VAH. The data from the above three studies do not support that down-regulation of dopamine mechanisms in the CB are responsible for VAH in the goat.

Thus, we are looking for still other mechanisms which could be responsible for increased CB sensitivity to hypoxia. CB dopamine function may be involved in another way, possibly as an excitatory agent. This is based on the finding of increased catecholaminergic metabolism during chronic hypoxia, primarily involving dopamine, that has been accumulating during the last few years. There is increased content and turn-over rate for dopamine in chronically hypoxic animals^{23,24}. Though this has not been adequately studied over the time course of VAH, there is evidence that increased tyrosine hydroxylase activity can be elicited in the CB after as little as one hour of hypoxia in rats⁹. This, in combination with a long standing hypothesis that dopamine might have an excitatory role in the CB¹⁶ has led us to consider the possibility that excitatory CB dopaminergic up-regulation may be a potential mechanism of VAH. We have found that the dominant effect of dopamine is inhibitory to the CB when it is given by intracarotid injection in the goat. However, in anesthetized goats with carotid sinus nerve recording, a brief burst of excitation is induced in afferent discharge of the CB upon bolus intracarotid injection of dopamine. This brief excitation is followed by a longer period of inhibition. It has been postulated that excitatory, but low affinity receptors for dopamine exist in the CB, but that it is difficult to readily elicit a response from these receptors with an intravascular infusion of dopamine¹⁶. It is suggested that, using low dose intravascular infusions of dopamine, it is not possible to reach the very high concentration of dopamine that is normally present during CB stimulation at the synaptic cleft between type 1 cell (releasing dopamine) and post synaptic receptor on the afferent nerve terminal¹⁶. Such a possible excitatory receptor has been postulated on the basis of excitation induced in *in vitro* CB preparations^{20,34} and after bolus infusions in dogs⁷ and now in goats. Studies are beginning to investigate the possibility that excitatory CB dopaminergic mechanisms could be involved in the mechanism of VAH.

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CHAPTER 19

ADAPTATION OF

O₂-CHEMORECEPTORS TO

HYPOXIA *IN VITRO*

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Introduction

Long-term exposure of humans and animals to hypobaric or normobaric hypoxia, e.g. high altitude or chronic lung disease, causes time-dependent changes in the hypoxic ventilatory response.^{1,10,19} In the case of animals born into and maintained in chronic hypoxia there is a blunting of the hypoxic ventilatory response.^{3,13} On the other hand, sustained hypoxia in mature animals can result in ventilatory acclimatization to hypoxia, where there is a progressive increase in alveolar ventilation.¹⁰ The mechanisms underlying these plastic changes in the ventilatory response during sustained hypoxia are not well understood, though there is evidence that peripheral chemoreceptors in the carotid body (CB) are involved. The CB contains O₂-chemoreceptors, i.e. glomus cells, which are thought to depolarize during acute hypoxia and release excitatory neurotransmitters onto afferent terminals of the carotid sinus nerve.⁴ The latter projects centrally to the respiratory control center in the brainstem, where its impulse activity regulates ventilation.

The changes in ventilatory response after chronic hypoxia are accompanied by both morphological and biochemical changes in the CB. For example, there is CB enlargement and this is associated with hypertrophy and possibly hyperplasia of glomus cells.^{2,8} In addition, the catecholaminergic properties of these cells are also modified, resulting in increased catecholamine turnover,¹⁴ and it is plausible that some of these biochemical changes may underlie 'blunting' of the hypoxic ventilatory response. Taken together, these observations point to glomus cells as key participants in the adaptive responses of the CB to chronic hypoxia *in vivo*. Though these cells are the recognized O₂-sensors, the cellular and molecular mechanisms that underlie their adaptive capabilities are difficult to investigate during chronic hypoxia *in vivo*, because of the complications of secondary cardiovascular adjustments. For example, in the intact animal chronic hypoxia causes blood changes in other CB chemostimuli, e.g. PCO₂ and pH,³ and in circulating hormones (e.g. glucocorticoid). Any of these are potential candidates for mediating the adaptive responses of glomus cells, rather than the direct stimulatory effects of low PO₂. To investigate whether direct exposure to low oxygen can lead to adaptive responses in glomus cells we have, over the last several years, used a cell culture approach where dissociated rat CB cells are grown chronically in an incubator, under an atmosphere

of reduced oxygen, but normal PCO_2 and pH. This strategy has uncovered several plastic properties in glomus cells that appear to result from their chronic stimulation by low oxygen.^{5,9,12,15,16}

Methods

Culture: The methods for growing dissociated carotid body cells from fetal and postnatal rat pups have been described in detail elsewhere.^{5,15,16} Briefly, the cells are grown on a thin layer of collagen in modified culture dishes, in serum supplemented F12 medium with various additives. To simulate chronic hypoxia the cultures are placed in a Forma Scientific automatic O_2/CO_2 incubator at 6% O_2 , 5% CO_2 (rest N_2) at 37°C; control normoxic cultures are grown in an atmosphere of 20% O_2 . In experiments where the role of growth factors (e.g. basic fibroblast growth factor; bFGF) is investigated a serum-free, chemically-defined medium is used.¹²

Electrophysiology: The perforated-patch, whole-cell technique was used to record whole cell currents (voltage clamp) or membrane potential (current clamp) from glomus cells grown in normoxia or chronic hypoxia *in vitro*. The recording methods, including composition of extracellular and pipette solutions, and procedures for data acquisition and analysis are discussed in detail elsewhere.¹⁵ The records shown in that text were obtained at room temperature in Hepes-buffered extracellular medium. To test for electrical excitability, depolarizing currents of increasing amplitude were injected into the cell in current clamp mode. To estimate glomus cell size, whole-cell capacitance (which is proportional to surface area) was determined from integration of the capacitative transient, recorded during hyperpolarizing voltage steps (voltage clamp).

Catecholamine determination: Basal and stimulus-evoked catecholamine (CA) release measurements were obtained from normoxic and chronically-hypoxic CB cultures using High Performance Liquid Chromatography (HPLC). The procedures were similar to those described elsewhere.¹⁷ At the end of release experiments an estimate of the number of glomus cells present was obtained following immunostaining of the cultures for tyrosine hydroxylase (TH), as previously described.⁵ This allowed CA release to be normalized to the number of TH-positive cells, and therefore, unlike *in vivo* studies, was not influenced by possible glomus cell hyperplasia.

Immunofluorescence: The procedures for immunofluorescent staining of the cultures for TH were similar to those previously described.⁵ To obtain an estimate of mitogenic activity (on glomus cells) of hypoxia and/or bFGF, acting alone or in combination, a double-label immunofluorescent procedure was used.^{11,12} In this assay, cultures received a 24-hr. pulse of the thymidine analog, bromodeoxyuridine (BrdU), and were processed for immunofluorescent detection of TH and BrdU using fluorescein and Texas-red conjugated secondary antibodies, respectively. The proportion of TH-positive cells, with nuclear BrdU labelling, provided an estimate of the fraction of glomus cells that entered the S-phase of the cell cycle during the 24-hr. BrdU pulse.

Results

Ionic currents and electrical excitability of glomus cells after chronic hypoxia:

Previous studies indicated that after CB cultures are exposed to chronic hypoxia (Chox) *in vitro* for 1-2 weeks there is modification of whole-cell currents in glomus cells. In particular, there was a reduced K^+ current density and an increase in peak

Na^+ and Ca^{2+} currents.^{15,16} Though there was some variability in the magnitude of the effect from cell to cell, overall there was a slight increase in Na^+ current density, whereas the Ca^{2+} current density was unchanged. Thus the increase in magnitude of the calcium current appeared to be due mainly to cell hypertrophy, as evidenced by the increase in membrane capacitance.¹⁶ We hypothesized that the cAMP signalling pathway might be involved in the augmentation of Na^+ current response, since this second messenger is elevated in glomus cells during acute hypoxia,¹⁸ and chronic treatment of normoxic (Nox) cultures with cAMP analogs, greatly enhanced Na^+ currents in these cells.^{15,16}

The overall increase in inward currents, and decrease in K^+ current density, in glomus cells after chronic hypoxia *in vitro* suggested an increase in electrical excitability. Consistent with this, while there was no obvious change in resting membrane potential (typically -40 to -50 mV) after chronic hypoxia, during routine injection of depolarizing currents pulses, action potentials with both Na^+ and Ca^{2+} components were more readily elicited. A typical example is illustrated in Figure 1, where injections of similar depolarizing current pulses failed to elicit action potentials in a Nox cell (Fig. 1A), but readily did so in a Chox one (Fig. 1B). Additional evidence that Chox glomus cells are more easily excitable was obtained during con-

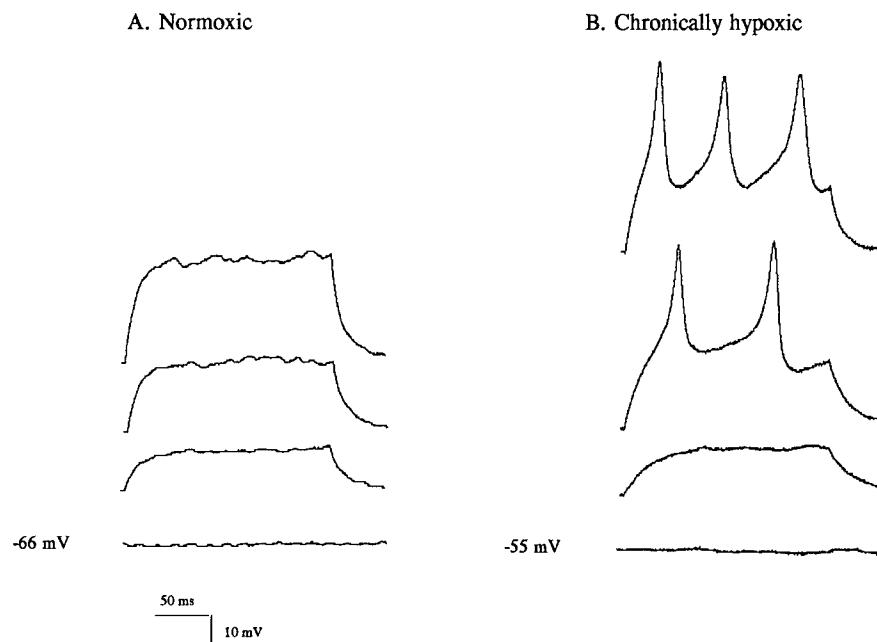


Figure 1 Effects of depolarizing stimuli on membrane potential of glomus cells grown under different oxygen tensions. In A, culture was grown under normoxic conditions for 15 days where, typically, increasing the magnitude of depolarizing stimuli failed to trigger action potentials in glomus cells at room temperature. Lower trace represents the zero current or resting potential (-66 mV); current was increased (upwards) in 10 pA steps. In B, culture was grown in chronic hypoxia for 12 days; note similar current injections readily elicited action potentials; this was a common pattern in chronically-hypoxic glomus cells. Input capacitance was 8.3 pF in A and 14.6 pF in B; the higher value after chronic hypoxia is due to cell hypertrophy.

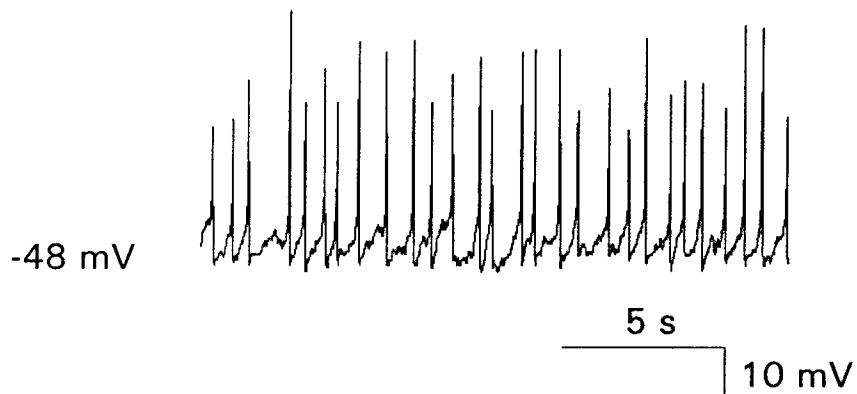


Figure 2 Spontaneous impulse activity in a chronically-hypoxic glomus cell. In this recording (at room temperature) the cell fired repetitively without current injection. Such cases rarely occurred in normoxic cultures, and is consistent with increased electrical excitability after chronic hypoxia. Culture was ~12 days in chronic hypoxia.

tinuous recordings of membrane potential. In several cases, for example Figure 2, Chox cells showed bursts of spontaneous action potentials (without current injection) during recordings at room temperature; such spontaneous activity rarely occurred in Nox cells.

Catecholamine release

Measurements of catecholamine release by HPLC revealed significant differences between Nox and Chox cultures. Perhaps the most notable was the elevated basal release rates in Chox cultures after 8-10 days exposure to 6% O₂ (Jackson and Nurse; *J. Neurochem.*; in press); basal release of dopamine (DA), normalized to the number of TH⁺ cells present, was found to be >6x higher in Chox cultures relative to 'sister' normoxic controls. Since release was normalized, comparison was not limited by differences in glomus cell number between cultures in either Nox or Chox environments. Thus, modifications in basal DA release after chronic hypoxia appear to be expressed at the level of individual glomus cells, and likely result from direct stimulation of these cells by low PO₂. One possible explanation is that the changes in membrane properties of glomus cells during chronic hypoxia (see above) result in higher spontaneous firing rates (Fig. 2), resulting in a greater influx of extracellular calcium, and enhanced DA secretion. Alternative explanations include a resetting of basal intracellular calcium to higher levels in Chox glomus cells, downregulation of DA re-uptake mechanisms, or enhanced stimulus-secretion coupling. As regards the latter, we previously observed in similar cultures an increased expression of the 'plasticity' protein GAP-43 in Chox glomus cells;⁵ though its physiological functions are still being investigated, GAP-43 is a calmodulin binding protein that has been implicated in promoting transmitter release from nerve terminals and neuroendocrine cells (see 5).

Control of glomus cell number by hypoxia and bFGF

One of the consequences of whole animal exposure to prolonged hypoxia is CB enlargement, which involves hypertrophy and possibly hyperplasia of glomus

cells.^{2,8} We have previously shown that chronic hypoxia *in vitro* causes glomus cell hypertrophy as reflected by a 3-4 fold increase in whole-cell capacitance and 3 dimensional cell volume.^{9,16} In recent studies we have found that treatment with KN-62 (4 μ M), an inhibitor of the Ca^{2+} /calmodulin-dependent protein kinase (CaM-kinase), can block the hypoxia-induced increase in whole-cell capacitance. Thus it is possible that this signal transduction pathway is involved in the glomus cell growth response during chronic hypoxia.

The question of whether glomus cell hyperplasia occurs during chronic hypoxia is still arguable.⁸ In a previous study, we observed that chronic hypoxia *in vitro* led to a significant increase in uptake of the thymidine analog, bromodeoxyuridine (BrdU), by glomus cells, without obvious effect on cell number.¹¹ This raises the possibility that glomus cells may undergo cell death after mitosis and may require other growth factors to support survival. In a recent study,¹² we obtained evidence

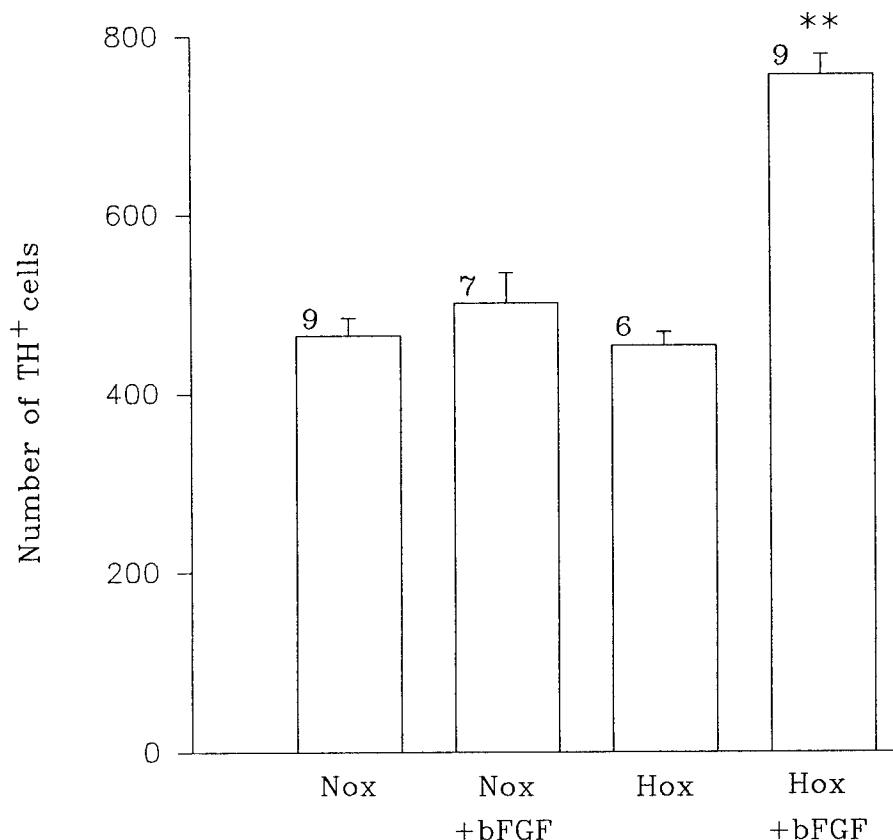


Figure 3 Effects of bFGF and hypoxia on number of surviving TH positive glomus cells. Cultures were plated at similar initial densities and transferred to serum-free, chemically-defined medium after the first 24 hr *in vitro* for an additional 48-hr, under each of the conditions indicated; normoxia (Nox), Nox plus 10 ng/ml bFGF, hypoxia (Hox; 6% O_2); or Hox plus 10 ng/ml bFGF. At the end of the treatment, cultures were immunostained for tyrosine hydroxylase in order to estimate the number of surviving glomus cells (vertical axis). Cell counts shown on histogram were similar to the initial density (i.e. at 24-hr. *in vitro*) for all treatments except, hypoxia plus bFGF, where there was significant ($p<0.01$) cell proliferation.

that basic fibroblast growth factor (bFGF) can collaborate with hypoxia to increase survival of perinatal glomus cells in serum free medium. Figure 3 illustrates this co-operative effect *in vitro* on glomus cells from 1-2 day-old rat pups. Interestingly, exposure of the cultures to bFGF or hypoxia alone for 48 hours had no detectable effect on the number of surviving TH-positive glomus cells. However, combined treatment with both bFGF and hypoxia caused glomus cell proliferation in 'sister' cultures, resulting in an (~1.5 fold) increase in cell number relative to the initial plating density. Since it appeared that both hypoxia and bFGF, acting alone, stimulated mitogenesis in glomus cells (based on increased BrdU uptake) but without proliferation,¹² their collaborative action may well be involved in the regulation of cell death. Our recent preliminary studies suggest that glomus cell death may occur in culture by an 'apoptotic' pathway based on positive nuclear staining with the TUNEL reaction.¹²

Discussion

Our studies are based on an *in vitro* model which attempts to uncover the cellular and molecular mechanisms involved in the carotid body chemoreceptor response to chronic hypoxia. Unlike the *in vivo* approach, where it is difficult to separate secondary cardiovascular effects due to chronic hypoxia, the model we have described allows, in principle, the direct effects of low oxygen on chemoreceptor (glomus) cells to be addressed. We have so far identified a series of adaptive changes at the cellular level, which may well contribute to the whole organ response to prolonged hypoxia. The *in vitro* model also permits potential signal transduction pathways to be explored, and we have evidence for the possible involvement of cAMP pathways in some of the adaptive responses.^{15,16}

Besides the observed changes in membrane currents and glomus cell excitability during chronic hypoxia *in vitro*,^{15,16} we have found that the basal secretory activity of glomus cells is increased, based on enhanced dopamine release. Since release of other CB neurotransmitters may be similarly affected after chronic hypoxia, the overall effect of carotid body stimulation on respiration will likely reflect the resulting balance of inhibitory and excitatory influences originating from secretory activity of glomus cells. It would not be surprising if blunting of the hypoxic ventilatory response and ventilatory acclimatization to hypoxia involve such changes at the level of glomus cells (see Introduction). A downregulation or reduction in functional O₂-sensitive Ca-dependent K⁺ channels has been found in acutely isolated glomus cells from rats born and reared in chronic hypoxia;²⁰ this may reflect yet another form of adaptation at the cellular level which may alter the O₂-sensing ability of these cells.

Finally, our recent studies on the interaction of the angiogenic growth factor, bFGF, and hypoxia in promoting proliferation and/or survival of perinatal rat glomus cells suggest novel mechanisms for regulating glomus cell number and carotid body size.¹² One possibility is that hypoxia may upregulate bFGF receptors in glomus cells, as it does in retinal pigment and vascular endothelial cells,^{6,7} and in this way modify their response to local or circulating bFGF. Release of the latter in low PO₂ conditions, e.g. from circulating macrophages,⁷ could provide a mechanism by which chemoreceptor number could be regulated by 'apoptosis' during *in utero* development, in the presence of a hypoxaemic fetal circulation.

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CHAPTER 20

VENTILATORY RESPONSE TO EXPERIMENTAL HYPOXIA IN ADULT MALE AND FEMALE NATIVES OF THE TIBETAN AND ANDEAN PLATEAUS

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Abstract

This paper reports the results of a comparative study of the ventilatory response to additional experimental hypoxia (hypoxic ventilatory response HVR) in adult native residents (20-94 years of age), (Aymara), living on the Andean (n=444) and Tibetan (n=210) plateaus (4000m). The study was designed to describe this component of cardiopulmonary physiology in adults of both sexes and to identify sex, age, body size, and population influences on HVR in the two populations. Tibetan HVR was roughly double that of Aymara. Age was not a significant contributor to HVR variation in either sample; there was no evidence of a decrease in HVR with increasing age. In each population, males had higher HVR than females ($p<0.01$) and between populations Tibetans of each sex had higher values than Aymara ($P<0.01$). Results of the present study suggest both high and low values for HVR are compatible with successful lifelong adaptation to high altitude.

Introduction

The ability to increase ventilation in response to lowered levels of environmental oxygen is a critical component of successful adaptation to acute hypoxia;³¹ however, the literature is uncertain as to ventilatory responses in those exposed to lifelong hypoxia or to years after a move to living at a high altitude. Adult residents living at 2700-3100m in the Andes and Colorado have a relatively small ventilatory response to further experimentally-induced hypoxia (hypoxic ventilatory response=HVR) when compared to newcomers.^{12,25,30} Studies of adult migrants in the Andes with periods of 3-42 years of residence at 3990 and 4515m also hypoventilate relative to newcomers.⁵ Adult migrants to 3100m in Colorado develop a depressed response to experimental hypoxia, inversely related to the length of high altitude residence.³⁰ Evidence that a low HVR can be acquired during childhood, adolescence, and adulthood led to the inference that a low HVR was a physiologic response to high altitude hypoxia.⁴ However, subsequent reports described values in young adult Sherpas in Nepal and Tibetans indicated that some individuals breathe and respond

to additional hypoxia no differently than newcomers.^{9,11,32} Thus, there may be no unique ventilatory adaptation to high altitude hypoxia that confers success.

The findings on the Tibetan plateau span a narrow age range and involved a limited number of subjects. In addition, there are methodological differences among Tibetan, Andean, and North American studies. Values for HVR obtained from small samples are subject to ascertainment bias and sampling errors, or may inadvertently be affected by environmental exposures, such as travel or migration history, household effects, or occupation(s). Demographic and physiologic information were not collected uniformly across studies. Finally, few studies have included women in the sample or have compared values between sexes. Therefore, the size and extent of population contrasts was not clear and the ability to incorporate results into a more general model of physiologic fitness remained limited. There are, however, differences in hemoglobin concentration and oxygen saturation at rest between Tibetan and Andean high altitude natives,¹⁻³ suggesting a need for further investigation of cardiopulmonary function and HVR in particular.

This paper describes the results of a study designed to describe HVR in large samples of males and females of Tibetan and Andean origin living at 4000m values, utilizing similar recruitment, data collection, and testing procedures.

Methods

The data described in this report were obtained on the Tibetan plateau in 1993 and on the Andean plateau in 1994. Verbal informed consent was obtained from all participants in accordance with procedures approved by the Committee on Human Subjects of Case Western Reserve University.

Subject selection: Tibetan participants were recruited in the Tibetan Autonomous Region, China. The study sites were two agropastoral villages with a population of 773 ethnic Tibetans living at 3800-4065m. The average barometric pressure was 479 torr. Each household was contacted in May, June, October, or November, 1993, in order to invite participants and their biological relatives 9 years or older to participate in the study. 96% of the households contributed 1 or more participants and 68% of those eligible by age participated to yield a total sample of 428 people, aged 9-82. Age was verified by reference to reported animal year of birth which was translated into Western calendar years. All were lifelong high altitude native residents at 3600m or higher, with the exception of one low altitude native who had lived in the village for 43 years.

Andean participants were recruited in four dispersed communities in Provincia Murillo, Departamento La Paz, Bolivia from a population of 1175 ethnic Aymara living at 3900-4000m. The average barometric pressure was 478 torr. Between May and August, 1994, each household in these communities was contacted and all household members and biological relatives aged 14 and older were invited to participate in this study. Over 75% of all households participated and 57% of age eligible residents participated. Seventy percent of the sample resided within the four communities of Provincial Murillo and the rest were relatives who resided elsewhere. All were Aymara (except 1 Quechua) natives of this or nearby high altitude communities. One relative resided at a lower altitude. Sixty-four percent of the participants' ages were verified by birth certificates or an identity card issued to the individual upon presentation of a birth certificate.

Data Collection. Demographic, lifestyle, and health information were obtained by an interview in the native language. Lifestyle information included information on genealogy, smoking history, alcohol and tea consumption, coca chewing, and occupational and migration history. Open and closed ended questions about health status, including questions to detect chronic cough, exercise intolerance, and tuberculosis, and for women reproductive status were asked. Nearly all households cooked with dung fuel in kitchens, that were physically separate from the main dwelling, or had a separate entrance. Andean highlanders were asked to abstain from coca chewing on the day of the test. Anyone who indicated that he had chewed coca that day or had fresh coca leaves in his mouth was asked to return another day. Physiological measurements and most anthropometric measures were taken by one of the authors by herself or by a local assistant under her supervision. Anthropometric measurements were taken according to a well developed protocol.^{1,2,3} Following the history and collection of anthropometric data, values of resting ventilation and ventilatory responsiveness were obtained. Before ventilatory measurements, resting oxygen saturation of arterial hemoglobin (SaO₂%) was obtained.

Hypoxic ventilatory responsiveness (HVR) challenge: In each population, the ventilatory response to additional experimental hypoxia was measured by the Rebuck and Campbell rebreathing technique,²¹ modified for the testing conditions in these rural communities. These modifications were: a) initiation of the re-breathing trial from a three-liter mixture of 5% CO₂ and 20-21% oxygen (rather than 5-6% CO₂ and 24% oxygen), b) termination of re-breathing trials at an oxygen saturation of 75% (rather than 65%), and c) slowing the rate of descent of oxygen saturation by adding supplemental room air. As in the Rebuck and Campbell technique,^{21,22} isocapnia was maintained at or near the average end-tidal value for the subject, as determined while the subject breathed at rest into the circuit, and the value for hypoxic ventilatory responsiveness was calculated as the change in ventilation per percent fall in oxygen saturation ($\Delta V_E/\Delta SaO_2$).

Prior to the protocol, the participant was familiarized with the testing procedures and practiced breathing with the mouthpiece and noseclips. The subject sat for 10-15 minutes before testing began. During the protocol the subject was seated, nostrils occluded with noseclips and breathing through a mouthpiece. Inspiratory and expiratory airflow velocity was measured by a Fleisch pneumatograph and a Validyne pressure transducer (+/- 2 cmH₂O). The output of the pressure transducer, amplified by a Validyne carrier demodulator, was used to determine ventilatory frequency and inspiratory and expiratory volumes. The transducer was balanced electronically and calibrated by a three-liter volume syringe with 1 liter increments before and after each subject.

Valves directed gases either to the room or to the rebreathing circuit. Once the study participant became accustomed to the apparatus, and appeared to be breathing comfortably, three minutes of resting ventilation and baseline values of oxygen saturation and end-tidal CO₂ were recorded. For re-breathing trials, the subject's exhaled gases were directed through the rebreathing circuit by large bore tubing and low resistance, one-way valves. Midpoint in the rebreathing circuit was a T-piece connector. Exhaled gas was directed through the circuit, a variable speed blower drew a portion through the T-piece connector and through a chamber containing carbon dioxide absorbing crystals to remove carbon dioxide. The gas was returned

into a seven-liter weather balloon, which was inflated with three liters of room air prior to each individual rebreathing trial. Thus, the subject re-breathed previously exhaled gas, some of which had been scrubbed of some carbon dioxide. The speed of the blower determined the flow through the scrubbing circuit and regulated end-tidal carbon dioxide. The rate of arterial oxygen desaturation during the rebreathing trial was slowed by an infusion of room air into the rebreathing circuit by an external pump. The goal was to slow the rate of arterial oxygen desaturation to 1-1.5% every fifteen seconds, permitting a slow decline in saturation to 75% over at least 90 seconds at a stable end-tidal CO₂.

During collection of baseline ventilation and each HVR trial, inspiratory and expiratory carbon dioxide (CO₂) were continuously measured at the mouthpiece, in real-time, by a Biochem Lifespan CO₂ monitor. The gas was monitored at the most proximal point to the subject on the expiratory side of the circuit and gas was recirculated at 150 cc/minute though the CO₂ monitor. The accuracy of the monitor was validated with a two point calibration, every 2-3 study participants.

Oxygen saturation was measured by a Criticare 501+ pulse oximeter. The oximeter's sensor was placed upon the subject's second, third, or fourth finger and values of oxygen saturation were displayed and updated every four to six heart-beats (approximately one to three seconds). The pulse oximeter also measured and displayed heart rate using photoplethysmography.

Computer hardware and software: The analog outputs from the CO₂ monitor, pulse oximeter, and the Validyne transducer amplifiers were connected to a Toshiba 486/33 MHZ portable computer that was equipped with a Data Translation 2815 analog to digital converter. Subject data was recorded with Vital Signals, a program designed to collect, analyze, graph, and store physiologic data. Specific physiologic parameters and recording frequencies were as follows: airflow velocity at 50 Hz, oxygen saturation 1 at Hz, inspiratory and expiratory CO₂ at 10 Hz, and arterial pulse waveform at 50 Hz. The computer stored the flow time values for volume calibrations (in volts) and calculated breath-by-breath values for inspiratory and expiratory tidal volume and minute ventilation.

HVR Criteria and Sample Selection. Three HVR challenges (each separated by a 10 minute rest period) were performed on each participant. Each record was reviewed for inclusion or exclusion in the final data and excluded from analysis if: a) less than 90 seconds of data were collected, b) breath-by-breath end tidal CO₂ fluctuated by more than 4 mmHg around the end-tidal value for the subject observed during resting breathing, c) a decrease of >40% occurred in the amplitude of the airflow signal (indicative of pneumotachograph failure), d) oxygen saturation acutely dropped more than 5% after the mouthpiece was in place, or e) if the subject or the investigator terminated the study before three trials were collected. HVR was calculated on a breath-by-breath basis as the change in minute ventilation divided by the change in oxygen saturation ($\Delta V_E/\Delta SaO_2$).

Statistical Analyses. The analyses reported here were conducted within a sub-sample (see above) of 210 Tibetans (88 males) and 444 Aymara (232 males) 20-94 years of age. Individuals 20 years of age and older are considered adults because height growth is completed by then in both sexes in both populations. At least one acceptable HVR was obtained in 210 Tibetans and 444 Aymara. In 79% of Tibetans and 92% of Aymara, there were collected three acceptable tests. In 5% of Tibetans and 1% of Aymara, only one HVR test met criteria for acceptability. Results from all tests in each subject were averaged to provide one value for each subject.

Table 1
 Characteristics of Adult Aymara and Tibetan Study Participants > 20 years of age
 (values represent mean +/- s.e.m.)

	Males		Females	
	Tibetan	Aymara	Tibetan	Aymara
Age (years)	38±2	40±1	38±1	40±1
Height (cms)	165±0.7	160±0.3	153±0.5	149±0.3
Weight (kg)	52±0.7	59±0.6	45±0.5	52±0.7
Body Surface area (m ²)	1.55±0.01	1.61±0.01	1.38±0.01	1.45±0.01
SaO ₂ %	88±0.4	92±0.2	89±0.3	91±0.3
HVR (L/min/%SaO ₂)	-0.97±0.01	-0.47±0.02	-0.72±0.06	-0.38±0.02
End tidal CO ₂ (mmHg)	30.1±0.6	33.3±0.6	29.4±0.5	34.0±0.6

Bivariate correlation and t-tests addressed various hypotheses. A significance level of 0.05 is used. Data values will be reported as the mean ± the standard error of the mean (S.E.M.).

Results

Table 1 describes the physical characteristics and physiologic data of the Tibetan and Aymara men and women in this sample. There were no differences in age between the men and women in each group. There were significant differences in anthropometric characteristics and values of oxygen saturation at rest. Tibetans were physically taller and lighter, resulting in a smaller body surface area, and had a lower oxygen saturation. The mean difference in HVR values was significant for both women and men in the two samples and between samples for each sex. End-tidal CO₂ values were significantly higher in women than men in each population, but there was no differences in values between populations of the same sex.

HVR was generally higher in the Tibetans than in the Aymara at all ages (Fig. 1). There was no statistical evidence of a decline with age, with the exception of Aymara males (Table 2).

Table 2
 Bivariate Correlations to Hypoxia Ventilatory Response (HVR)

	Tibetan		Aymara	
	Males	Females	Males	Females
Correlations with:				
Age (years)	+0.02	-0.05	-0.18*	-0.14
Height (cm)	0.00	-0.09	+0.15*	+0.14
Body surface area	-0.02	+0.08	-0.15*	-0.12
Chest width (cm)	+0.16	+0.09	+0.19 *	+0.17 *
Chest depth (cm)	-0.06	-0.02	-0.05	+0.01
Resting SaO ₂ (%)	-0.11	-0.14	+0.02	+0.25

* p < 0.05

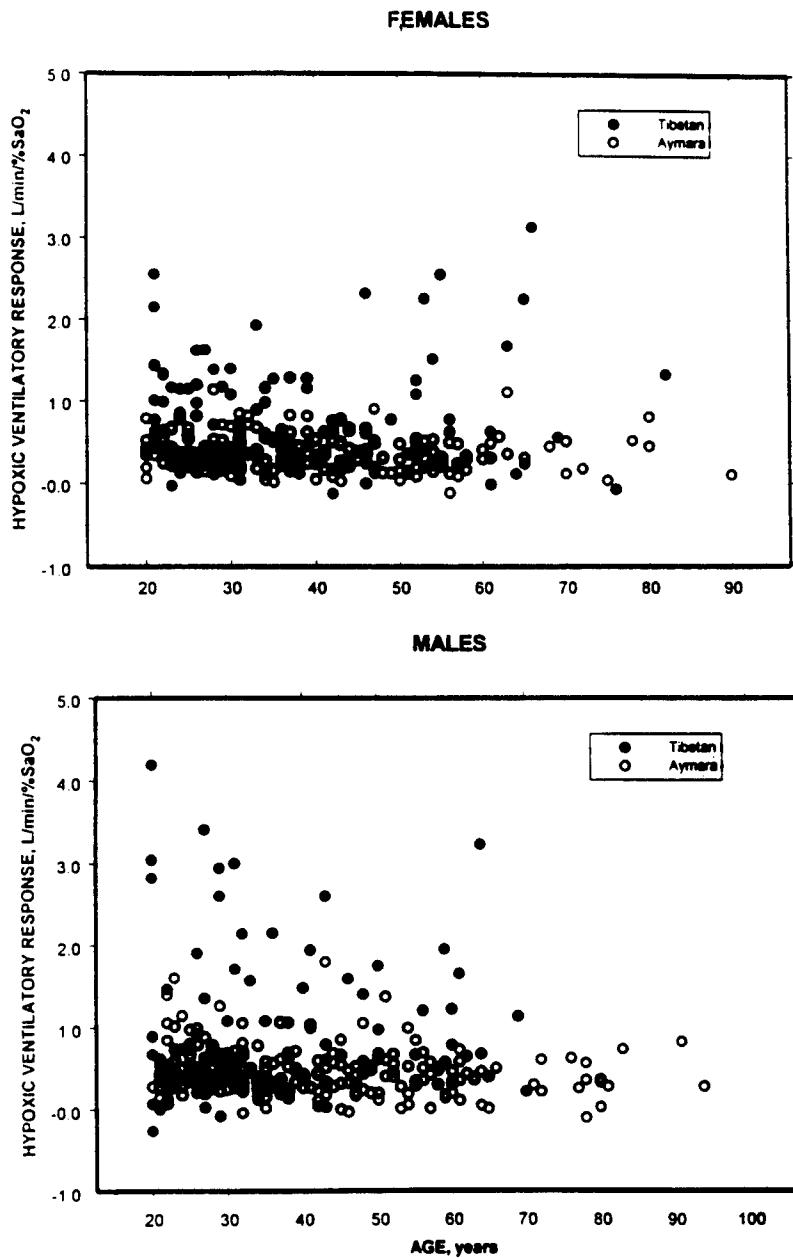


Figure 1 Hypoxic ventilatory response (HVR) (L/min/%SaO₂) of Tibetan and Aymara males and females by age.

Figure 2 displays the data on HVR relative to body surface area. The HVR values for Tibetans are generally higher than the Aymara throughout the range; there was no statistical correlation with body surface area, with the exception of Aymara males (Table 2).

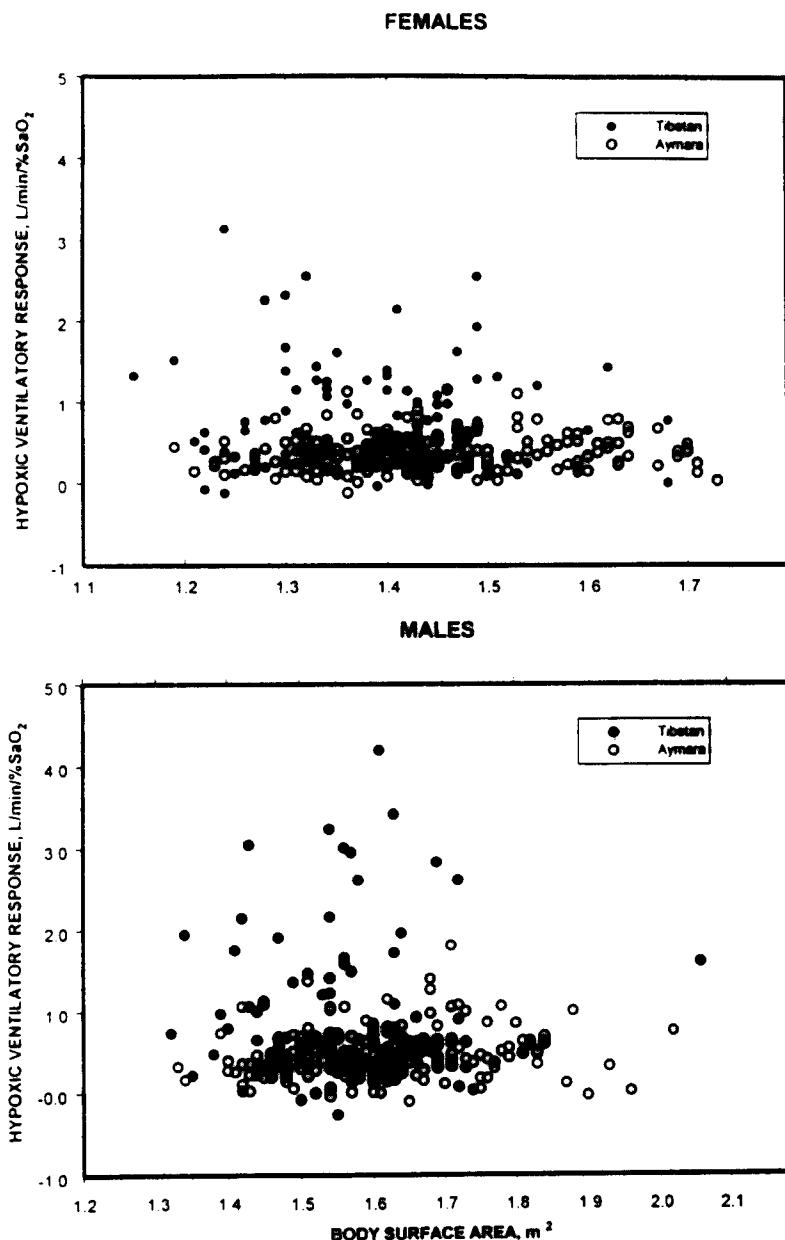


Figure 2 Hypoxic ventilatory response (HVR) of Tibetan and Aymara males and females by body surface area (m^2).

Table 2 shows the bivariate correlations between HVR and body size and shape. HVR was inversely correlated with age in the Aymara men, but the correlation accounted for about 5% of the variance in HVR. HVR was correlated with body surface area (m^2) only in Aymara males, and the association explained a small

proportion of variance. Height and chest width, independently, exhibited slight positive correlations and a small association with HVR in the Aymara but, not the Tibetan, sample. HVR did not correlate with SaO_2 among Tibetan men or women or in Aymara men, but had a low positive association among Aymara adult females.

Lifestyle factors are not associated with significant variance in HVR. While most Tibetan adults drank salted butter tea, the volume consumed (categorized as < 1 L/day, 1-2 L/day or 2+ L/day) was not associated with HVR. Among Aymara adults there was no effect on HVR of chewing coca leaves. Among Tibetan men (Tibetan women do not report smoking), there is no smoking related difference in Tibetan HVR. Similarly, there is no influence of smoking on Aymara HVR.

Discussion

These data demonstrate that healthy Tibetan males and females had a substantially higher mean increase in ventilation upon exposure to additional experimentally induced hypoxia when compared to an Andean sample of Aymara. When exposed to additional hypoxia, Tibetan breathing increased roughly twice as much as the Aymara. A 10% drop in SaO_2 , with no change in carbon dioxide levels, produces about a 9.3 L/min increase among Tibetan men compared to a 4.5 L/min increase among Aymara men. The population difference was as large or larger than the sex difference in each population and occurred from the 3rd through the 9th decade of life.

The study was designed and implemented to exclude differences in recruitment, measurement and analyses as sources of variance. The results confirm and extend observations on smaller samples of young adult men. Figure 1 summarizes reports of HVR in 805 adult natives and long term residents above 2000m, including 654 from the present study, and contrasts these with reports from studies at lower altitudes. There is a range of variation in values for HVR at sea level and all but one of the high altitude samples lie within that range. Five of the six Tibetan samples lie in the middle of the sea level range of variation while one is in the low end. The present Andean sample lies in the lower end of the sea level range and the one other study of Andean highlanders reporting HVR in this form reports a mean below the sea level normal range.¹⁸ That study was consistent with other studies reporting that both Andean and Colorado high altitude natives have very low HVR compared to sea level natives.^{8,26} In general, adult Asian high altitude natives have a mid-range HVR and Andean high altitude natives have a low HVR, compared with sea level.

Five neurobiological components can account for the generation of a behavior, like HVR, that will occur in response to an external stimulus; these are hormonal processes, genetics and developmental biology, neurophysiology, decision processing, and psychophysiological constraints.²⁰ Hormonal factors, including sex steroids could account for differences among men and women^{17,23} but are unlikely to contribute to the difference among populations. Another factor like this is metabolic rate, which is known to affect HVR,^{10,23} but it appears that both Tibetan and Andean highlanders have basal metabolic rates similar to those predicted by sea level equations.³ Endurance training can be associated with differences in ventilatory responsiveness,²² however, it seems unlikely that this occurs on a population-wide basis throughout the life cycle or explain the sex differences, for in both populations women perform agricultural tasks throughout the year and men also are active in occupations that also require fitness.

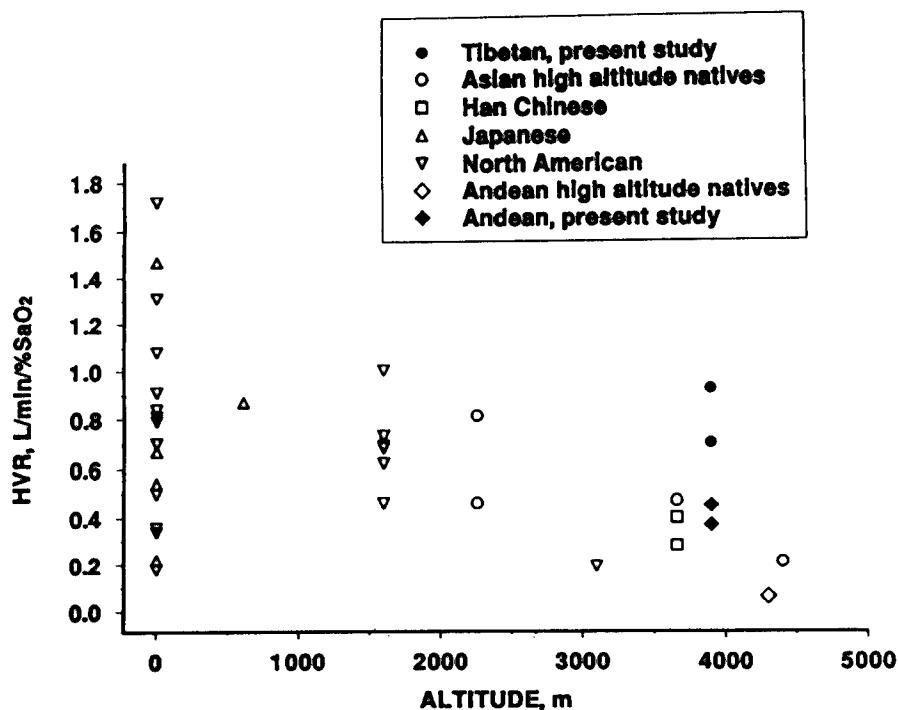


Figure 3 Summary of mean values from studies reporting ventilatory response to experimental hypoxia according to various altitudes.

In regard to development, there is evidence that neonatal exposure to respiratory stimuli can either blunt or enhance HVR^{7,27} and intrauterine cocaine exposure is associated with blunted HVR in infant humans.²⁹ In animals, neonatal exposures to high or low oxygen will result in long-lasting changes in respiratory behavior.^{14,19} Lahiri et al¹³ proposed such a process might operate in regard to ventilatory adaptation to altitude. We have no evidence to argue that these factors account for the variance of HVR between Tibetans and Aymara. A clustering of HVR values, especially depressed values, can occur in families of patients with chronic cardiopulmonary disease;¹⁶ however, it seems unlikely that a chronic disease process explains an Aymara population wide phenomenon. More likely is the operation of familial transmission of traits like HVR.^{6,16,22}

There is a component of higher cognitive functioning in any behavior during wakefulness, and the measurement of HVR is subject to inherent experience and psychophysiologic factors.^{7,22} The presentation of the study and the testing itself was similar in each community, making it less likely that the equipment induced a response that was systematically different. That does not mean that the response of each population was similar in regard to learning or perception. We have no data to address the potential action of these influences.

Hypothetically, differences in HVR could reflect different diurnal exposures to hypoxia, as might occur during sleep or with activity-inactivity profiles. There is little data as to population contrasts in these areas; the data we collected on the Andean

plateau suggested that a low HVR is accompanied by normal sleep in young or older men.³

If the principle force determining a physiologic behavior like HVR is the action of natural selection, a wide range of values in high altitude populations appears to be accompanied by success. A lower HVR does not appear to have adverse consequences for the Aymara nor does a higher value appear to provide an advantage for the Tibetans, since both populations are expanding in numbers. In regard to other physiologic adaptations, however, the Tibetan and Aymara samples vary in regard to components involved in oxygen delivery.¹⁻³ The results of the present paper add credence to the speculation that natural selection has acted to produce well adapted, but distinct phenotypes in the two geographic areas.

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CHAPTER 21

DEVELOPMENTAL ASPECTS OF THE VENTILATORY ADAPTATION TO HYPOXIA: HYPOMETABOLISM AND ITS IMPLICATIONS

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In mammals, *hyperventilation*, defined as the increase in ventilation relative to the metabolic level,* represents one of the most immediate and important defense mechanisms against acute hypoxia.

In man and many mammals of medium to large size, hyperventilation in most cases results exclusively from *hyperpnea*, defined as an increase in the absolute level of \dot{V}_E (and \dot{V}_A). On the other hand, in mammals of smaller size, and in many young or newborn species, including the human infant,⁴ hyperventilation is also contributed by hypometabolism; in fact, in many cases, hypoxic hyperventilation is solely due to a decrease in metabolic level, with no changes or a reduction in \dot{V}_E .^{7,26,27}

This presentation will focus on the main features of the hypometabolic response to hypoxia during the neonatal period, with reference to its potential implications on the control of ventilation.

Postnatal development.

In the newborn, hypometabolism is often the only contributor to hypoxic hyperventilation. Later, with growth and development, hypometabolism becomes a less prominent component of the hyperventilatory response to hypoxia, whereas hyperpnea eventually prevails (Fig. 1). The reasons and the mechanisms behind this developmental pattern are unclear, and probably reflect a combination of many factors. What seems to be a general pattern is that the hypometabolic response is more apparent the higher the normoxic metabolic level. For example, among adult mammals, those with higher normoxic $\dot{V}O_2$ (per unit of body weight), usually the smaller species, have the larger metabolic drop in hypoxia. Equally, when $\dot{V}O_2$ is

*In its application to CO_2 , hyperventilation implies an increase in alveolar ventilation (\dot{V}_A) relative to carbon dioxide production (\dot{V}_{CO_2}); hence, a decrease in alveolar (and therefore arterial) P_{CO_2} [$P_{ACO_2} = (\dot{V}_{CO_2}/\dot{V}_A) \cdot Pb$, where Pb is barometric pressure, dry].

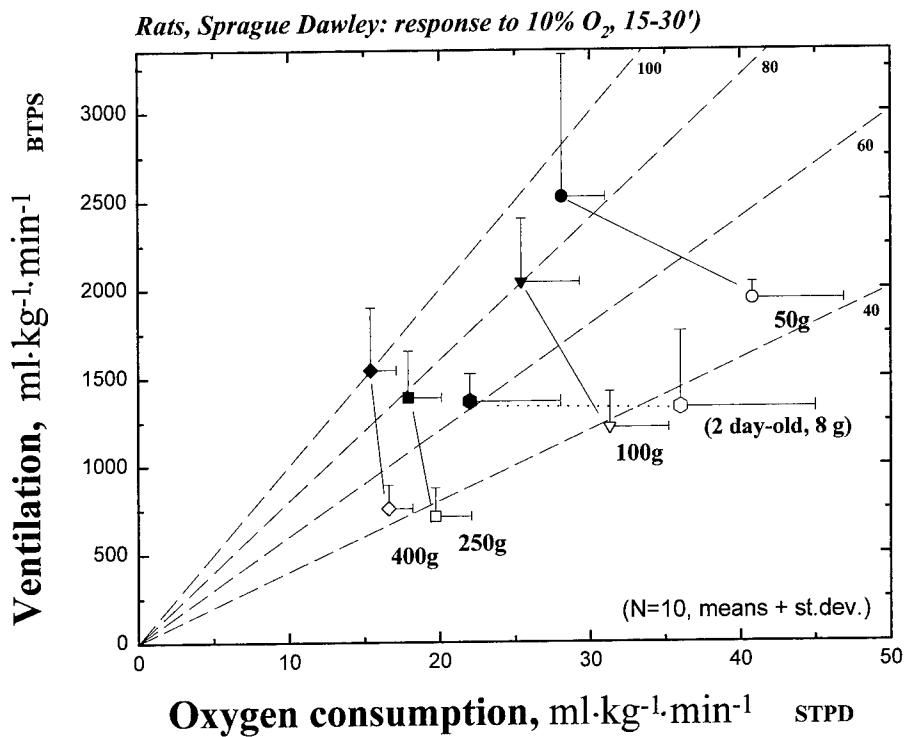


Figure 1 Oxygen consumption ($\dot{V}O_2$) - ventilation ($\dot{V}E$) relationships for groups of rats of different age and body mass. In normoxia, as the animal gets older, its $\dot{V}O_2/kg$ at first increases, then progressively drops (open symbols), and $\dot{V}E$ follows this developmental pattern quite closely. In hypoxia (10% O_2 breathing, 15-30 min, filled symbols), the younger the animal, the greater its hypometabolic response and the smaller the hyperpnea. (From the data of ref. 14,24).

increased by cold-thermogenesis, hypoxia evokes an hypometabolic response even in those animals which in warm conditions would not decrease $\dot{V}O_2$.^{19,40} Newborns have invariably higher $\dot{V}O_2/kg$ than adults, and this may contribute to their propensity for hypometabolism.

Because in mammals, including newborns, the level of resting normoxic $\dot{V}O_2$ is largely determined by thermogenic requirements, the above and other considerations suggested that hypoxic inhibition of thermogenesis is the major mechanism responsible for the reduction in metabolic rate,^{8,16} in agreement with what first emerged from the work of June Hill.¹² In addition, in young animals, the inhibition of cell repair, tissue growth and differentiation are other functions curtailed by hypoxia, and their inhibition is a likely contributor to the hypoxic reduction in $\dot{V}O_2$, with no activation of anaerobic energy sources. The consequences of the inhibition of these processes on body growth and cardiorespiratory development become clearly apparent with prolonged or chronic hypoxemia.^{5,25,28,36,43}

Hypoxic specificity.

An acute increase in inspired CO_2 (2-5%) and the parallel arterial acidemia do not result in hypometabolism (Fig. 2). This indicates that hypometabolism in

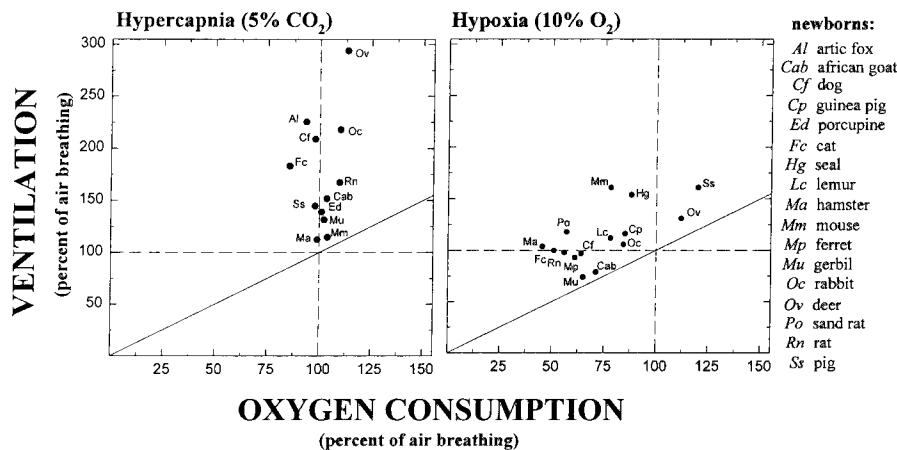


Figure 2 Oxygen consumption ($\dot{V}O_2$) - Ventilation ($\dot{V}E$) relationship in several newborn species. Each symbol is the average value, expressed in percent of the value in air. During hypercapnia, differently from hypoxia, the metabolic change is minimal, and the hyperventilation is strictly by hyperpnea. (From the data of ref. 21,27).

newborns is not an undifferentiated response to any form of chemoreceptors stimulation. Indeed, from studies in adult animals, it is known that the presence of the peripheral chemoreceptors is not necessary for the hypoxic reduction in $\dot{V}O_2$, and that hypometabolism can also occur when blood O_2 content is reduced with normal PaO_2 , as in anemia or carbon-monoxide poisoning, i.e. in hypoxic conditions which do not activate the carotid chemoafferents.¹⁹

Addition of CO_2 to the inspired air during the hypoxic condition increases $\dot{V}E$ without appreciable modification of the hypometabolic response,^{23,39} indicating that mechanical factors within the respiratory system and muscles do not represent a constraint limiting the degree of hypoxic hyperpnea, and 'forcing' the newborn toward the hypometabolic strategy. Indeed, newborn rats have been shown to be capable of prolonged and sustained hyperpnea, as during several days of normoxic hypercapnia.³⁷

Ambient and body temperature.

Because the hypoxic inhibition of thermogenesis (which in newborns is mostly, if not exclusively, non-shivering thermogenesis of the brown fat) is likely to be a major contributor to the reduction in $\dot{V}O_2$,^{8,11} hypometabolism is more apparent in cold conditions than close to, or at, thermoneutrality (Fig. 3).

Precisely how hypoxia interferes with non-shivering thermogenesis is not known. During neonatal chronic hypoxia, the mass of the Brown Adipose Tissue (BAT) and the concentration of mitochondrial uncoupling protein *thermogenin* decrease (unpublished measurements). In acute conditions, hypoxia could interact with non-shivering thermogenesis by changes in regional blood flow,¹¹ or by action on the BAT adipocytes, either directly or via neural control through the hypothalamus, although no direct information is available.

With the decrease in metabolism, body temperature (T_b) also decreases, by a magnitude which depends largely on the species size. In fact, heat dissipation, in first

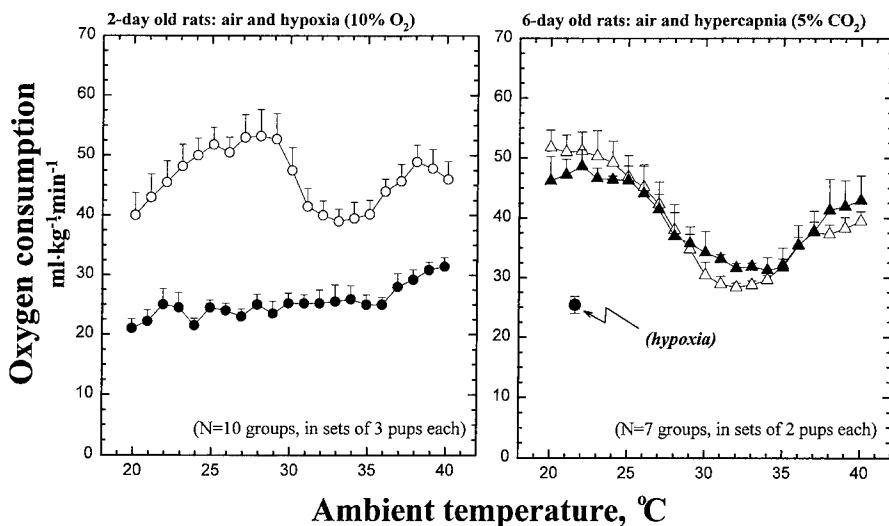


Figure 3 Ambient temperature - Oxygen consumption relationship in rat pups. At left, the pups (2-day old) were exposed to air (open circles) or hypoxia (filled circles). At right, the pups (6-day old) were exposed to air (open triangles), hypercapnia (filled triangles), and eventually to hypoxia (filled circle, arrow). Symbols represent mean values, bars, 1 SEM. (From the data of ref. 18,42).

approximation, depends upon body surface, which, relatively to body mass, is smaller in larger species. In adult rats it has been shown that hypoxia not only decreases thermogenesis, but also lowers the animal's preferred temperature and the set-point of thermoregulation.^{6,10,13} This phenomenon has not been conclusively established in newborns, but some considerations suggest that it may occur. For example, in hypoxic kittens, artificial warming to increase T_b to the normoxic value stimulated ventilation and decreased systemic vascular resistance (Fig. 4), responses which could reflect the kitten's attempt to heat-dissipate. If indeed normal T_b in hypoxia was perceived by the hypoxic newborn as an hyperthermic condition, it could lead to responses (such as an increase in cardiac output and redistribution of the blood to the periphery) which may conflict with its main strategies against hypoxia, which consist in energy saving and oxygen delivery toward the vital central organs. The advantage of a low temperature in the hypoxic newborn, and its physiological basis, have been often pointed out,^{6,15,33,38,41} but they are usually overlooked in the management of the hypoxic infant. The issue of 'relative' hyperthermia of the newborn during hypoxia could also be relevant to the understanding of abnormalities of the breathing pattern.

Coupling with pulmonary ventilation.

During acute hypoxia, despite the variety of metabolic conditions and responses both within and among animals, the degree of hypoxic hyperventilation is rather uniform. In fact, in general, the larger the hypometabolic response to hypoxia, the lesser the hyperpneic response. In newborn puppies, for example, during warm and cold exposure the ventilatory and metabolic responses to hypoxia were very different, but the degree of hypoxic hyperventilation (drop in $PaCO_2$) was almost identical (Fig. 5).

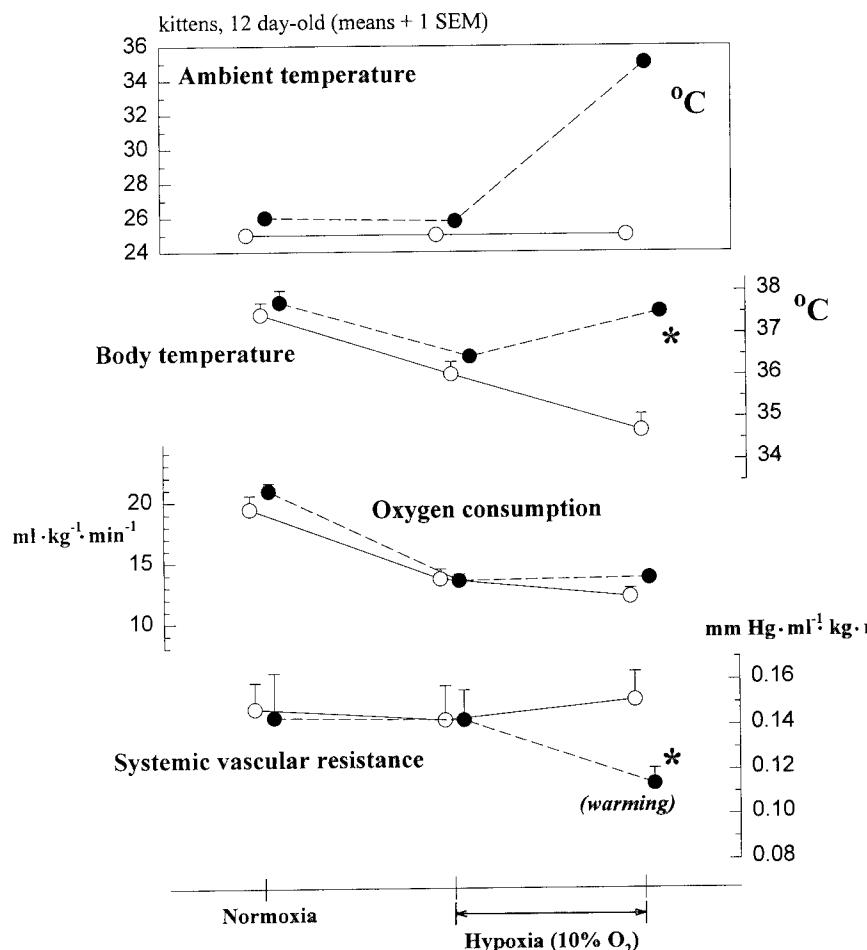


Figure 4 Conscious kittens were exposed to hypoxia, and in some of them (filled symbols) body temperature was raised to the normoxic values by artificially increasing ambient temperature. Artificial warming during hypoxia did not appreciably modify oxygen consumption, but did drop systemic vascular resistance. *, significant difference between the two groups. (From the data of ref.38).

This uniformity in the degree of hyperventilation, irrespective of the magnitude of the hypometabolic response, reflects the coupling between metabolic and ventilatory rates.⁸ Isocapnic, and quasi-isocapnic hyperpnea is well known to occur in several conditions of *increased* metabolic rate, such as pharmacological interventions, muscle exercise, thermogenesis, or during circadian oscillations in metabolic rate. The ventilatory-metabolic coupling during hypoxia can therefore be seen as an extension of this remarkable association in the direction of hypometabolism.^{17,19}

What are the mechanisms maintaining the metabolic-ventilatory coupling in hypermetabolic conditions is not known, although the possibility of an involvement of the CO₂ metabolically produced (VCO₂) has often been raised.^{19,34,46} A tonic CO₂ drive has also been considered important for the spontaneous breathing activity during fetal life.² In the newborn, during hypoxic hypometabolism, the possibility that

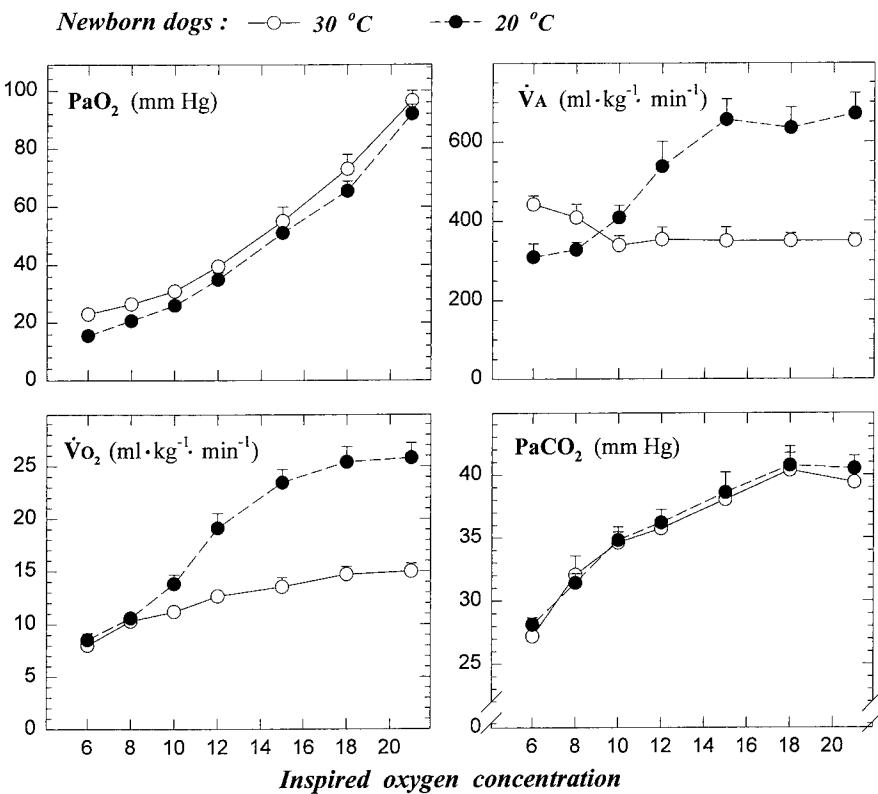


Figure 5 Conscious newborn dogs (11 days old, 675 g), breathing progressively lower oxygen concentrations (F_{1O_2} , from 21% to 6%, in 15 min. steps) during warm (30°C, open symbols) or cold (20°C, filled symbols) ambient conditions. The metabolic (oxygen consumption, \dot{V}_{O_2}) and ventilatory (alveolar ventilation, \dot{V}_A) responses to hypoxia were very different between the two temperatures, but the degree of hyperventilation (decrease in arterial PCO_2) was the same. Symbols indicate mean values of 13 animals, bars are 1 SEM (from Rohlicek CV, Matsuoka T, Saiki C and Mortola JP, unpublished data).

\dot{V}_{CO_2} played a role in controlling the ventilatory level is supported only by indirect observations. For example, exogenous administration of CO_2 can drastically modify the ventilatory response to hypoxia, from absent, to adult-type hyperpnea, without altering gaseous metabolism. Hence, one interpretation of the small hyperpneic response during acute neonatal hypoxia could be that during hypoxic hypometabolism \dot{V}_{CO_2} is reduced, and because CO_2 is an important facilitatory stimulus on ventilatory drive, its reduction results in a lowering of the ventilatory level. Whether a reduction in the metabolic drive *per se*, in addition to possibly controlling the ventilatory level, may also affect the sensitivity to ventilatory stimuli, and in particular the ventilatory sensitivity to hypoxia and hypercapnia, has not been directly assessed. In the adult rat, the ventilatory response to CO_2 does not seem to depend on the metabolic level,^{9,22} but similar experiments in the newborn have not been done.

Ventilatory inhibition during hypoxic hypometabolism

The possibility of a reduction in the metabolic drive to breathe during neonatal hypoxia raises the question of whether inputs normally inhibitory on breathing may

increase their relative efficacy. In other words, the hypothesis could be made that the normal balance between facilitation and inhibition on the respiratory controller may be disrupted if a stimulant (metabolic CO_2) is reduced, with relative amplification of inhibition. One attempt to examine this hypothesis was done by measuring the strength of the Hering-Breuer reflex in conscious newborn rats at different ages. In the newborn rat, chemosensitivity changes rapidly with postnatal development, being almost absent at day 2, and progressively more evident at day 5 and 8. Hypoxia clearly reduced the strength of the reflex in the 8-day old pups, as it was expected from experiments on adults, since any increase in chemical stimuli is known to reduce the pulmonary vagal inhibition.^{3,35,45} On the other hand, at the younger ages during hypoxia the reflex was either unaltered or even magnified. One interpretation that could be given to these results is that when the ventilatory chemosensitivity is low, a further reduction in ventilatory drive by the hypometabolism has the capacity for enhancing the relative efficacy of the pulmonary vagal inhibition. Whether similar events could apply to other reflexes inhibitory on neonatal breathing, for example reflexes of laryngeal origin, is not known.

In summary, it seems that some data are available to at least entertain the possibility that in the acutely hypoxic newborn the hypometabolic response can reduce the ventilatory drive, possibly by a reduction in the endogenous CO_2 , creating the basis for a greater efficacy of ventilatory inhibition (Fig. 6). When this sequence is coupled to special associated circumstances, like low chemosensitivity, or hyperthermic conditions (or, as discussed previously, an hyperthermic condition as perceived by the hypoxic newborn), then, it would seem to have the potentials for becoming a vicious cycle, which could have irreversible inhibitory effects on breathing.

Hypometabolism: why?

If the above possibilities have any plausibility, we may then ask why, in acute hypoxia, would some mammals, and newborns in particular, opt for hypometabolism instead of hyperpnea, putting themselves at the risk of being closer to the edge of

Neonatal hypoxia:

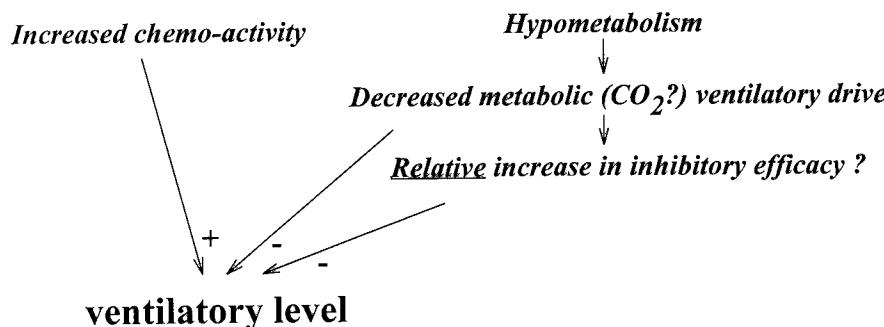


Figure 6 Events accompanying neonatal hypoxia. The *relative* greater efficacy of inhibitory inputs (right-hand side) would seem to be a potential basis for a vicious cycle which, by progressive aggravation of the hypoxia, could lead to irreversible apnea.

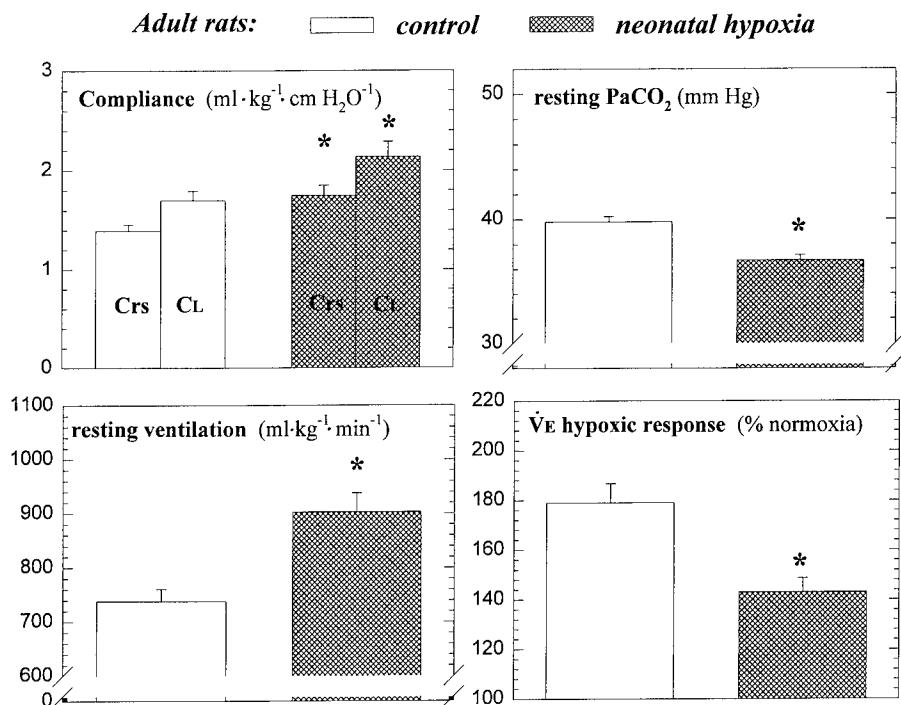


Figure 7 Adult rats exposed to hypoxia in the neonatal period ($\text{F}_{\text{IO}_2}=10\%$, from day 1 to day 6) still presented several alterations of the respiratory structure and function, among which, a larger compliance of the lung (C_L) and of the respiratory system (Crs), some degree of resting hyperventilation, and a blunted response to acute hypoxia, compared to rats never exposed to hypoxia. These differences are reminiscent of the characteristics of the human highlander. Values are means \pm 1 SEM. *, significant difference between the two groups. (From the data of ref. 30,31,32).

ventilatory inhibition. The answer to a teleological question can only be speculative. Perhaps, the newborn in hypoxia is still retaining the approach adopted during the fetal life, in which an hyperpneic response to hypoxia would only be a futile energetic loss. Perhaps, in the neonatal period hypometabolism is a more reliable strategy against hypoxia than any attempt to increase convection via cardiorespiratory mechanisms, which are undergoing major mechanical and regulatory adjustments to the novel air-breathing condition. Or it could be one more example of the general philosophy of Nature of reducing the use of a metabolic substrate when it is perceived to be scarce, as during mammalian hibernation or estivation, an approach so successfully and widely applied against hypoxia by a large variety of living creatures. Whatever its basis, the hypometabolic response is one of the factors responsible for the enormous and renowned capabilities of the newborn in resisting and recovering from severe hypoxia or anoxia.^{1,29,41,44} Its major consequences become apparent when the hypoxic condition persists and transforms itself into a chronic situation. The reduction in cell growth and tissue differentiation, and the fact that some organs are affected more than others according to their metabolic requirements and the protection that they receive by blood flow redistribution, lead to uneven growth and development, which may not be compatible with survival.²⁰

When the pup does survive, or when the chronic hypoxia resolves, the body undergoes a process of *catch-up growth* which seems to re-establish the proper interorgans relations. However, by use of animal models, a number of long-term alterations have been recognized, which, with respect to the respiratory function, include alterations in pulmonary and respiratory system mechanics, in pulmonary circulation and in the control of breathing. Of interest are the observations that adult rats chronically hypoxic in the neonatal period retained some degree of hyperventilation in normoxia, presented a blunting of the ventilatory response to a new acute hypoxic episode, and had an increased lung compliance (Fig. 7); none of these changes were seen in rats which experienced chronic hypoxia after weaning age. This triad of respiratory features (some normoxic hyperventilation, high compliance, blunted response to hypoxia) is recognised as a characteristic of the human highlander who has been living at high altitude for generations. Hence, laboratory data on animal models would indicate that the highlander's features do not need to be taken as genetic characteristics, but may reflect long-term effects of hypoxia, especially if the hypoxia began in the early developmental phases.

In conclusion, hypometabolism is a very common feature of the neonatal response to hypoxia. The mechanisms regulating it are under study, with much attention to the interaction between hypoxia and thermoregulation. The major advantage of hypoxic hypometabolism is survival against severe hypoxic or anoxic episodes, which would not be tolerated otherwise. The disadvantage emerges when the hypoxia becomes chronic, since the consequences of the decreased development may be irreversible and eventually incompatible with survival. Of major interest are the implications of neonatal hypoxic hypometabolism on the control of ventilation, which would seem to include the potentials for breathing irregularities and fatal apnea.

Acknowledgements

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CHAPTER 22

THICK BLOOD WITH THIN AIR: WHO NEEDS IT?

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The historic mining town of Leadville, Colorado high in the Rocky Mountains at an altitude of 10,150 feet (3,100 meters), is the highest incorporated community in North America. The Tabor Opera House standing on Main Street is a monument to the silver baron H. A. W. Tabor and his ill-fated wife Baby Doe.¹⁵ In more recent years, molybdenum replaced silver, and the Climax Molybdenum Mine astride Fremont Pass at 11,300 feet (3,400 m) became the economic base for Leadville with a stable population of about 8,000 persons. However, in the last decade, American steel has been replaced by imported foreign steel, the molybdenum mine has closed, and the population has dropped to about 3,500.

Thirty five years ago, when we first began investigating the effects of chronic hypoxia on the residents of Leadville, 'thick blood' was a common problem. 'Thick blood' was the term used by the residents to describe the excessive polycythemia affecting approximately 10% of men living and working in the Leadville-Climax area. But since an increase in hematocrit and hemoglobin concentration is considered an integral part of adaptation to high altitude, just when does this become 'excessive'? What are the normal limits of polycythemia at an altitude of 3,100 m where the average arterial oxygen tension is 65 Torr and the saturation is 89%? For this special population largely of European extraction and having resided at high altitude for no more than four generations, no data existed.

We set out to define the normal polycythemic response for this population. first in adults,⁹ by screening 210 volunteers. Of these, 64 men and 72 women were judged to be clinically normal. To survey the younger population,¹⁴ with the assistance of the Public Health Nurse, we obtained blood samples from the entire school population in grades 4 through 12. This consisted of 210 boys and 164 girls aged 10 to 18 years. From this total, 19 were excluded for medical reasons. In sum, 491 persons, male and female, 10 to 72 years of age were examined. Prior to puberty, hematocrit increased progressively with age with no sex difference (Fig.1). In females, by age 13 hematocrit reached a plateau at about 44%, whereas in males, hematocrit continued to rise, reaching the adult plateau of 50% by age 18. We saw no evidence of a progressive rise with increasing age among adult men as has been described at higher altitudes in the Andes.¹⁷

While this sex difference is well known, it does raise the interesting teleological question, do males have a physiological 'need' for a higher hematocrit than females? Clearly prior to puberty males and females function normally with equal levels of hematocrit. It is with the appearance of testosterone that red cell production is given

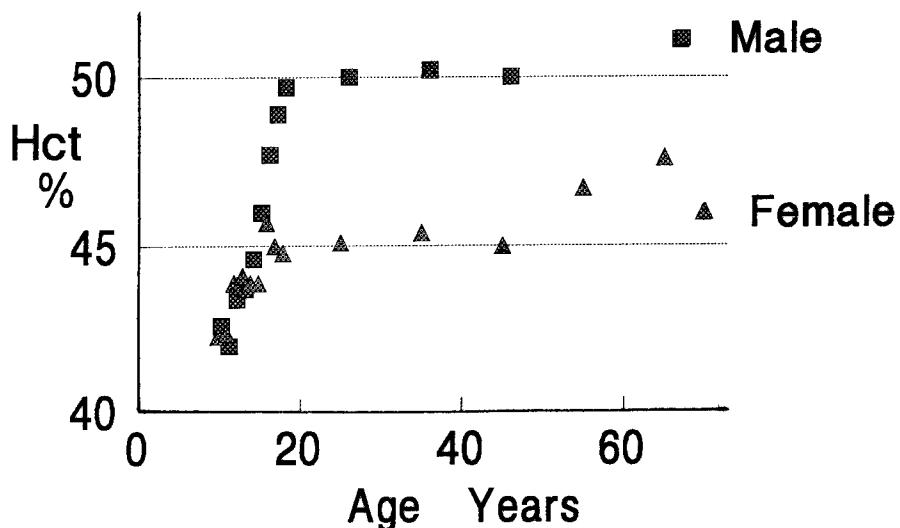


Figure 1 Mean hematocrit by age for male and female residents of Leadville, CO (n=491). While there is no sex difference prior to puberty, adult men as a group have higher values than women. Data from ref. 9 and 14.

an added stimulus, but is the resulting polycythemia merely a by product of testosterone, or are the demands for oxygen transport somehow different in adult men compared with adult women? And is this sex difference necessary for normal function? If we examine the adult data without regard to age, we find that the normal range, i.e. 95% of observations, for women is 40 to 53%, and for men 46 to 58%. This means that half of all men in Leadville have hematocrits no higher than observed in women (Fig. 2). Since there is no evidence these men are relatively anemic, the data strongly question any physiological 'need' for men to have higher values than women.

As to the question of when polycythemia becomes 'excessive,' the answer for men appears to be when the hematocrit exceeds 58%. For women, an hematocrit higher than 53% would be considered abnormal. However, the symptomatology of 'thick blood' probably reflects impairment of blood flow, particularly to the brain,¹³ resulting from increased blood viscosity, and such impairment becomes evident only when the hematocrit exceeds 58%. This would be equally true for both men and women.

To assess the magnitude of the polycythemic response to high altitude in the Leadville population, these data should be compared with normal blood values from sea level. In 1982 Fulwood et al.⁴ published an impressive summary of data collected from over 15,000 male and female residents of the United States. As would be expected, at all ages in both males and females, mean values for hematocrit are greater at the higher altitude (Fig. 3). For example, in adult women, the mean and normal range for hematocrit in Leadville are 45.5 (40-53)% compared with 40.5 (35-46)% at low altitude (Fig. 4). Note, however, over half of Leadville women have an hematocrit within the normal range for sea level. Similarly, in adult men, while the mean and normal range are elevated in Leadville, one third of these men have an

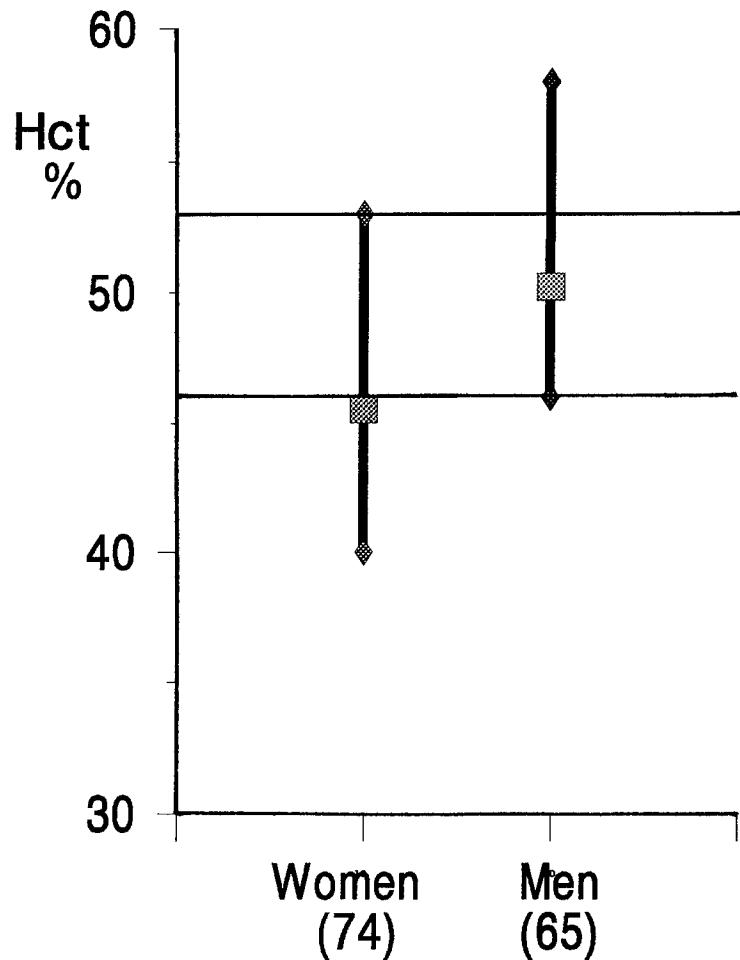


Figure 2 Among adults in Leadville, half of all men have hematocrits no higher than observed in women. Mean and normal range. Horizontal lines indicate range of overlap. Same data as in Fig. 1, from ref. 9.

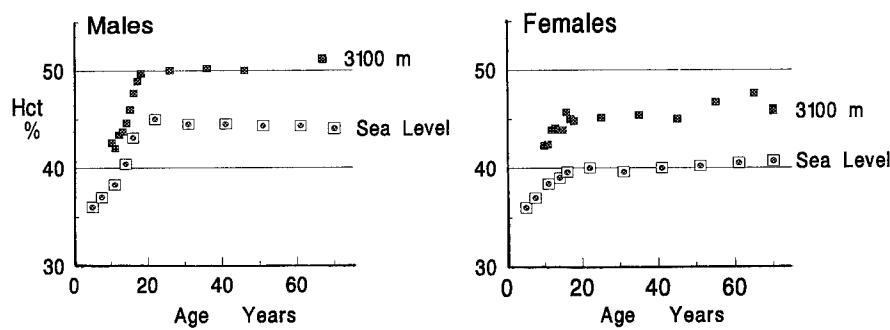


Figure 3 Mean hematocrits are higher at all ages at 3100 m altitude in Leadville than at sea level for both males (left) and females (right). Data from ref. 4, 9 and 14.

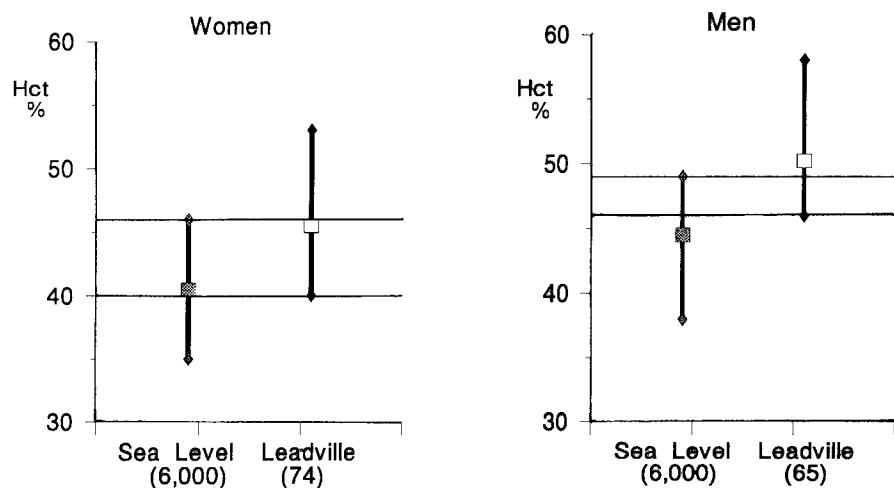


Figure 4 Among adults, one half of all women (left) and one third of all men (right) in Leadville have hematocrits no higher than observed at sea level. Mean and normal range. Horizontal lines indicate range of overlap at the two altitudes. Same data as in Fig. 3, from ref. 4 and 9.

hematocrit that is normal by sea level standards (Fig. 4). In other words, using hematocrit as the criterion, half the women and a third of the men in Leadville do not have polycythemia. Thick blood; who needs it?

To evaluate the absolute polycythemic response to high altitude, one should examine total body red cell volume. Using the same personnel, techniques and equipment, Weil et al.¹⁶ measured red cell volume in normal male residents at sea level and in Leadville. As expected, the mean value was greater at the higher altitude, 31.8 ± 6.6 (SD) vs. 27.1 ± 3.7 ml/kg. However, when one takes into account the wide normal range at high altitude, one third of men in Leadville have red cell volumes no greater than observed at sea level (Fig. 5). In other words, using the more rigorous criterion of an elevated red cell volume, one third of normal men fully acclimatized to residence at 3,100 m altitude do not exhibit a polycythemic response. Once again we say 'thick blood; who needs it?'

With such a sizable segment of the high altitude population having no increase in RCV or hematocrit, you begin to wonder if those who do have an increase show a 'benefit' from it. Does an increase in hematocrit convey some functional advantage? In the short term, the answer appears to be 'yes.' When healthy young men spent two weeks on the summit of Pikes Peak at 14,200 feet (4,300 m), hematocrit rose from 46% to 53%. Isovolemic reduction of hematocrit to 48% reduced arterial oxygen content by 10%, resulting in a lowering of aerobic working capacity ($VO_2\text{max}$) by 9%.⁷ Clearly, then, the higher hematocrit of 53% was advantageous, since working capacity was significantly impaired when hematocrit was lowered. However, still higher hematocrits may not convey additional 'benefit.' Sarnquist et al.¹¹ studied four mountaineers at 17,800 feet (5,400 m) whose hematocrit had risen to 58%. By our criteria, they had 'thick blood.' By means of isovolemic hemodilution, hematocrit was lowered to 50%, but in spite of a 13% reduction in blood oxygen carrying capacity, $VO_2\text{max}$ was not reduced significantly. Presumably improved blood flow consequent to lower blood viscosity maintained oxygen transport. Clearly excessive polycythemia can be too much of a good thing.

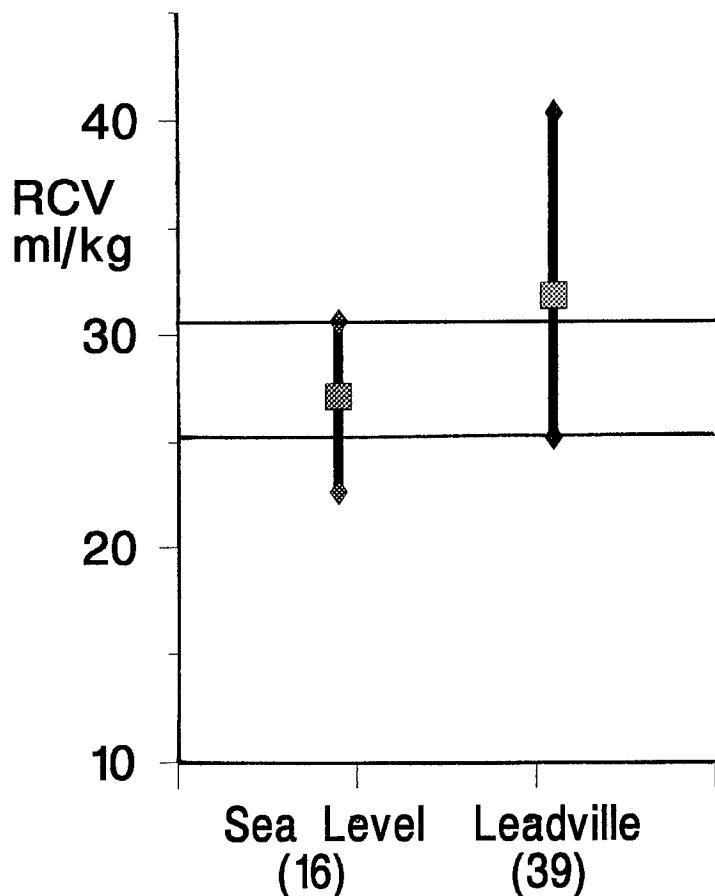


Figure 5 One third of all men in Leadville have red cell volumes (RCV, ml/kg) within the normal range for sea level. Number of subjects in parentheses. Data from ref. 16.

Up to this point, we have examined the changes in blood volume that have developed over years of residence at high altitude. How long does it take for the newcomer from low altitude to attain the values seen in long-term residents? What is the time course of changes in blood volume and its components, plasma and red cell volumes following ascent to high altitude? To answer these questions, normal men and women living near sea level must be transported to some mountain location where serial measurements can be made during the process of adaptation. For brief sojourns of 2 to 3 weeks, this has been accomplished by several investigators. However, for more extended periods, the logistic problems become formidable. As a consequence, data beyond 3 weeks are extremely limited. Furthermore, group mean data fail to convey the marked individual variability in the hematologic response.

Published reports containing data on individual male subjects adapting to altitudes of 4,000 to 4,500 m have been collected in Figure 6. At least four aspects of these data are apparent. Most striking is the inter-individual variability; the data resemble a 'scattergram.' Second, most investigators report a number of individuals who appear to have an initial *decrease* in red cell volume rather than no change or

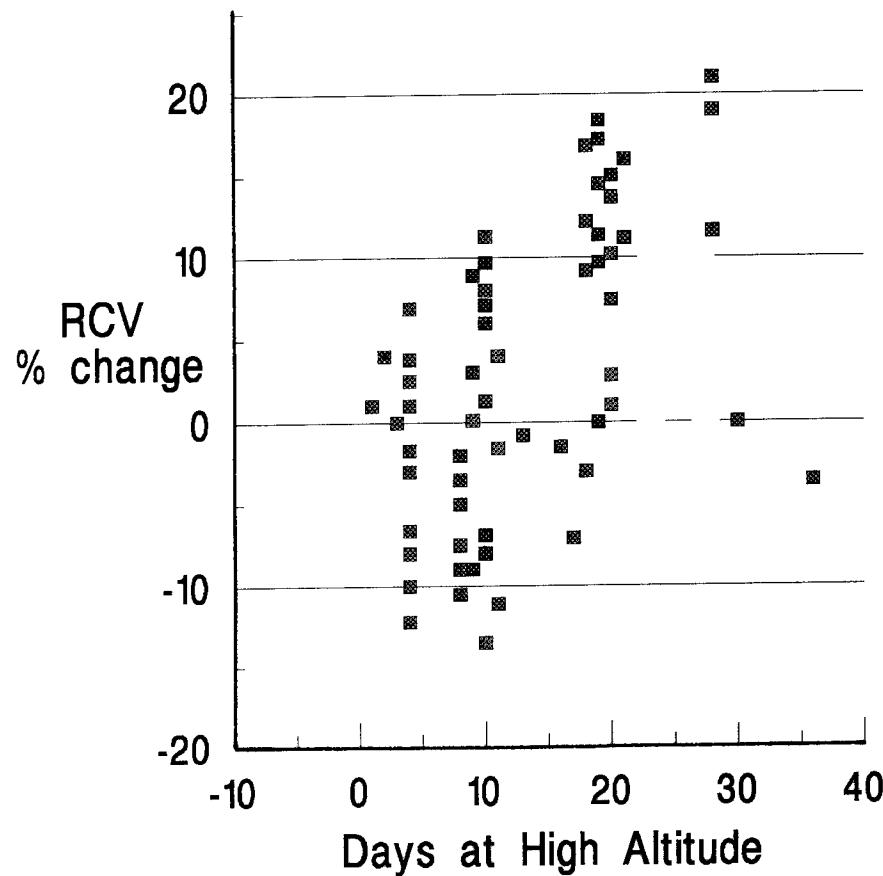


Figure 6 Time course of change in red cell volume (RCV, ml/kg) in men during the first month following ascent to altitudes from 4000 to 4500 m. Values for individuals expressed as % change from sea level. Data from 1, 3, 8, 12, 18, and 5.

an increase. Third, a clear increase in red cell volume of greater than 10% does not appear until the third week at altitude. And finally, there is the occasional normal individual who shows no measurable increase in red cell volume even after a month at altitude.

Only a single published report contains data covering adaptation over the first year at high altitude. The Peruvian investigator Cesar Reynafarje¹⁰ had 10 healthy young men in the military assigned to duty in the mining town of Morococha at an altitude of 4,540 m for one year. His group mean data demonstrate that red cell volume increases progressively over the first eight months, reaching a plateau at 50% above sea level. Plasma volume was still decreased about 20% during the second month, but recovered steadily thereafter. Consequently, total blood volume began increasing after the second month, and was 25% greater by the end of one year.

Changes in blood volume in women during acclimatization to high altitude have rarely been measured. Hemoconcentration which increases hematocrit and hemoglobin concentration, i.e. relative polycythemia, appears to proceed at the same rate

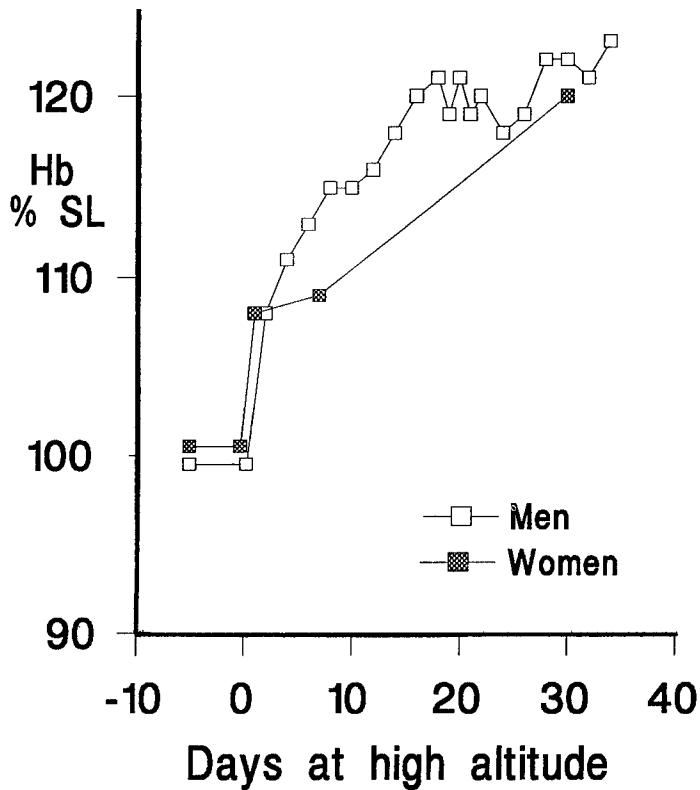


Figure 7 Time course for increase in hemoglobin concentration following ascent to 4300 m is the same for women⁶ as for men.² Data expressed as % of sea level values.

in women as in men (Fig. 7). The classic study published by Hannon et al. in 1969⁶ examined the development of true polycythemia, i.e. the change in red cell volume. Eight young women from Missouri spent 10 weeks on the summit of Pikes Peak employed in the visitor center. Upon ascent, red cell volume decreased approximately 10% and then gradually increased but never exceeded pre-ascent values. Plasma and blood volumes also remained decreased (Fig. 8). The authors concluded 'the hematopoietic response to altitude is markedly less in women than that usually observed in men.' However, exactly the opposite conclusion is indicated by data collected in 1996 by Moore et al. (personal communication), namely, the hematologic response proceeds at a more rapid pace in women than in men. Despite this uncertainty in the blood volume data, there is absolutely no doubt that women perform very well at high altitude.

The foregoing observations remind us that in the circulatory adaptation to the atmospheric hypoxia of high altitude, an increase in the oxygen carrying capacity of the blood, i.e. polycythemia, is but one component of the entire oxygen transport system. As with most physiological systems, the body employs multiple strategies to achieve a given result. To increase oxygen transport, alterations occur in heart rate, stroke volume, cardiac output and its distribution, the affinity of hemoglobin for oxygen, oxygen extraction from the blood, to name a few. Consequently, we should

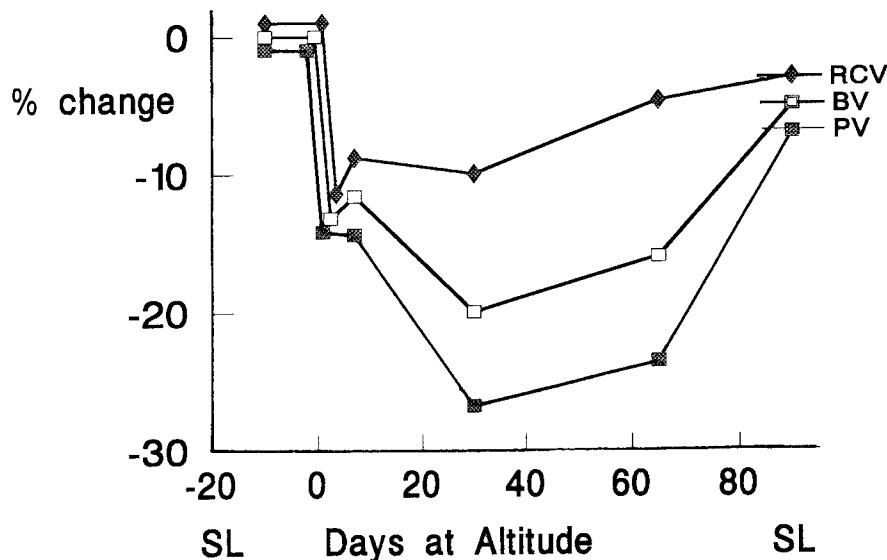


Figure 8 Time course of changes in red cell volume (RCV), plasma volume (PV), and blood volume (BV) in 8 women during a 10 week sojourn at 4300 m, and following return to sea level. Data expressed as % change from preascent values. Data from ref. 6.

not be surprised at the variability among individuals in the magnitude and time course of the polycythemic response, and the fact that in some cases there is little if any increase in red cell volume or hemoglobin concentration (hematocrit).

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CHAPTER 23

JOHN SUTTON AND ALTITUDE RESEARCH A STUDY IN HIGH ENERGY 1941-1996

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... the studies at altitude were always John's main love; when he wasn't above 15,000 feet he was planning the next trip, and this provided the main driving force for the science...

Norman Jones, personal communication, 1996

Introduction

Scientific work exercises the mind, but begins in the heart. John Sutton's heart was at high altitude, where oxygen availability is low, the environment is harsh, and exertion is essential. The picture of his scientific career, is painted with the broad strokes of energy utilization when body energy requirements are high, and the oxygen supply is limited. The broadest stroke which characterizes the overall picture of his altitude interest is the transport of oxygen from the air to the cellular mitochondria where the oxygen is burned to give energy. Nearly all his work dealt in some way with this fundamental issue. The proof lies in his bountiful publication record, (Fig. 1), which demonstrates that of some 270 publications, more than 95% dealt with some aspect of exercise and/or altitude. Even those concerned with sleep often had an altitude component. Sutton's heart was in energy production, especially at high altitude.

Sutton's earliest work already drew the outlines of his future career. His very first publication,¹⁵ itself a 'tour de force', asked the question, "Why do persons with high exercise capacity have slow resting heart rates?". In 1967, the conventional wisdom was that training decreased epinephrine output from the adrenal gland and also increased neural parasympathetic tone, both of which would lower heart rate. If so, blocking both β adrenergics (with propranolol) and the parasympathetics (with atropine) would, by freeing the heart from both influences, demonstrate an intrinsic heart rate which should then be independent of exercise capacity. But it was not so. In more than 70 normal men (Fig. 2) there was a broad range of intrinsic heart rates, where the lowest rates were in those with the highest exercise capacities. Further, Sutton showed prospectively that training lowered intrinsic heart rate. Subsequent animal research has confirmed that training effects occur despite β and parasympa-

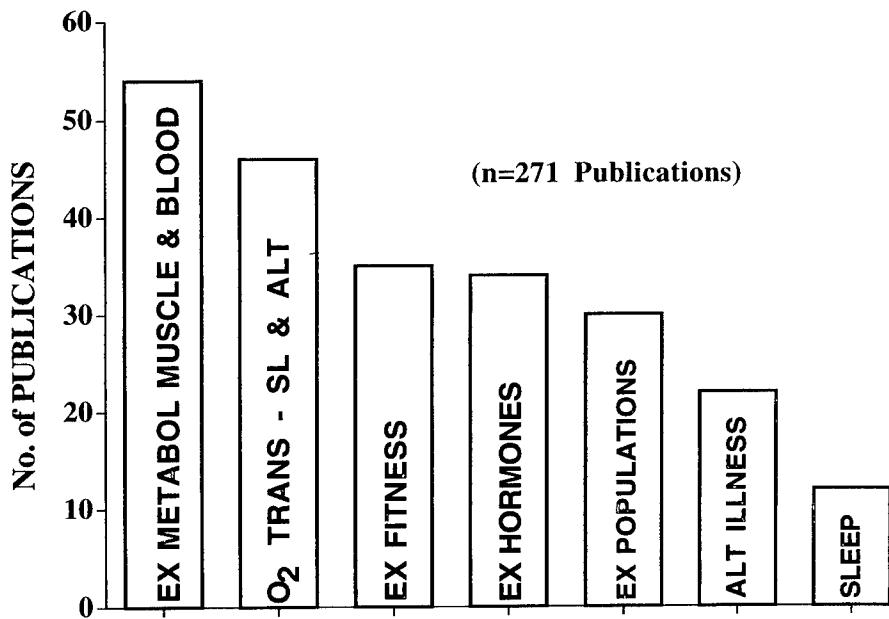


Figure 1 Categories of 271 papers authored by Sutton, published or in press. The categories from left to right, are exercise metabolism in either muscle or blood, oxygen transport at sea level and/or at altitude, exercise fitness, exercise hormone studies, exercise studies in populations under a variety of environmental conditions, altitude illness, sleep studies.

thetic blockade, but not when cardiac sympathetic nerves have been cut. John's initial research thus pointed the way toward fundamental issues of exercise capacity.

The early reports also already indicate the energy which Sutton brought to his projects. Because his first study involved so many subjects, the actual data must have been collected in 1965 and '66, when he was still in clinical training as a medical officer (Fig. 3). Further in 1969, when he had the combined duties of both medical registrar and a research fellow, he led the 9 member Australian Andean Expedition to Peru. Both research publications which came out of the expedition contained research as well as clinical data.^{13,14} Thus in his early career, from 1965-9 he was already balancing clinical and research duties while simultaneously developing his interests in exercise and high altitude. Somehow he found time as a traveling fellow to found the Performance Laboratory at the University of Sydney. The increasing responsibilities of his rapidly progressing career were associated with augmented scientific output (Fig. 3), indicating no diminution with time in his investigative zeal. Clearly his enthusiasm was infectious, because while he had some 70 solely authored publications, the great majority were with collaborators (Fig. 4).

Operation Everest II

Of the persons who collaborated with Sutton most frequently, nearly all were heavily involved in the enormous research project designated "Operation Everest II" (OE II). OE II was a project conducted in 1985 that repeated for the first time a similar but smaller study called Operation Everest sponsored by the US Navy in 1946.⁶ In OE II 8 healthy male volunteers were decompressed over some 40 days to the

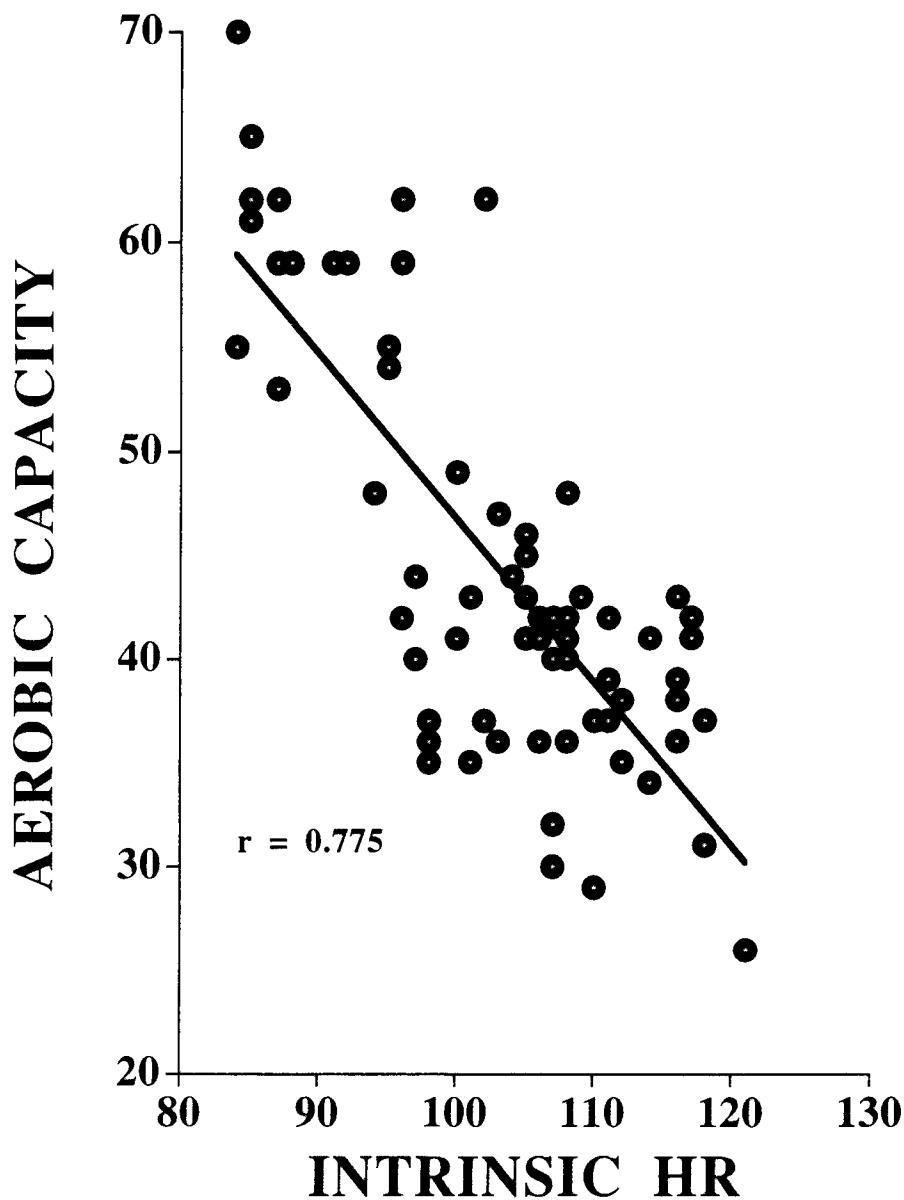


Figure 2 Aerobic capacity in 73 healthy young men as related to intrinsic heart rate. A strong negative correlation ($p<0.01$) indicates that more fit individuals have lower heart rates in the absence of β sympathetic or parasympathetic influences. (Redrawn from Sutton et al, 1976)

equivalent of the summit of Mt. Everest in the US Army Research Institute of Environmental Medicine chamber in Natick, MA.⁵ While the three primary organizers, Sutton, Houston and Cymerman, shared all aspects of the planning and conduct of the study, scientific responsibility fell more heavily on Sutton while Houston and

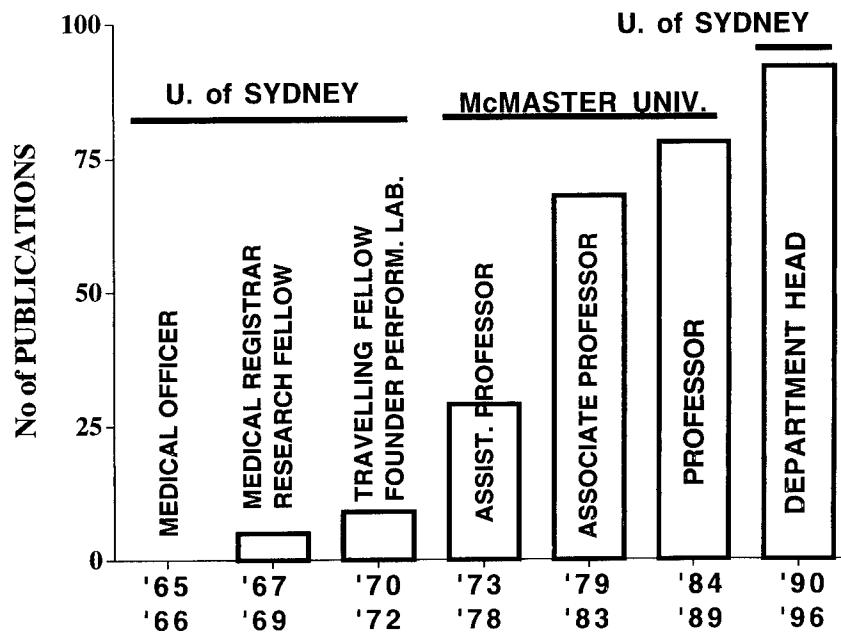


Figure 3 Time line of publication activity for Sutton from his beginning clinical training as a house officer in 1965 to his position as Departmental Head at the University of Sydney.

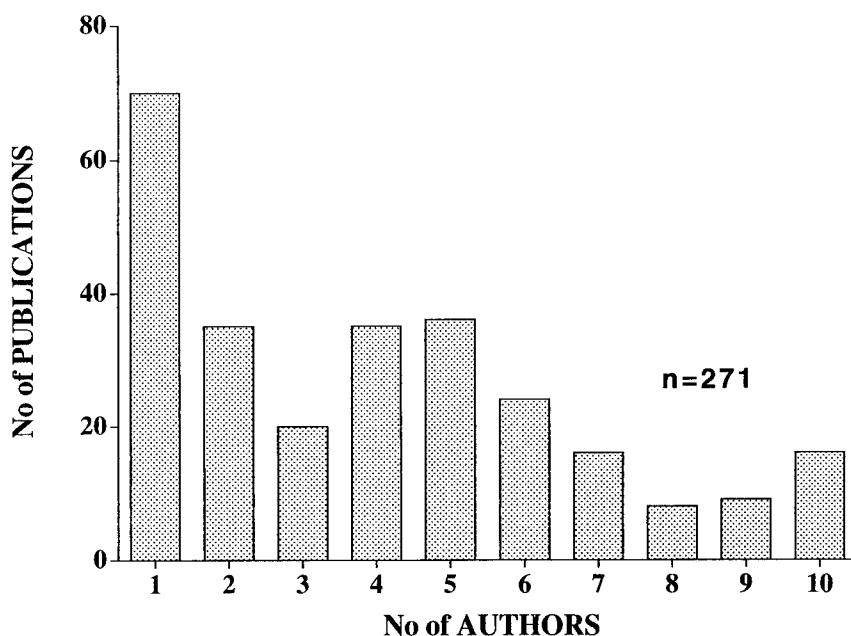


Figure 4 Shown for 271 publications by Sutton are the number of publications with him as sole author and the number with 2 through 10 collaborating authors. The publications with the higher number of collaborators, were often from Operation Everest II.

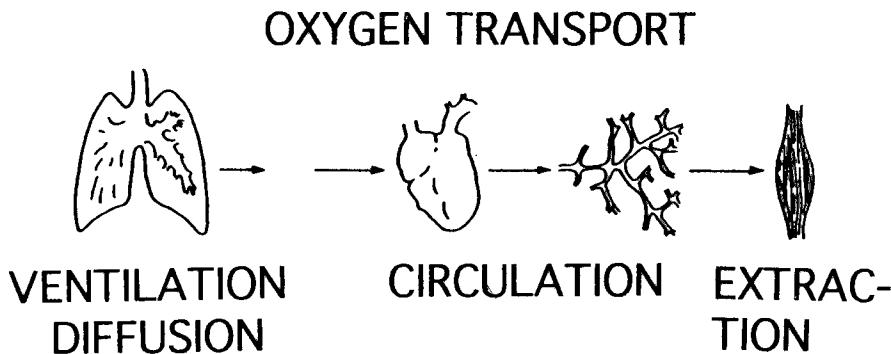


Figure 5 Sutton's schema for the oxygen transport cascade as modified from reference 17.

Cymerman were necessarily concerned more with administration and facilities, respectively. In a very real sense, OE II may be considered the ultimate manifestation of Sutton's research interests because it involved exercises to the limit of human capacity, exposures to near the limit of human low oxygen tolerance, studies of oxygen transport to most of the essential body organs, and collaboration with some two dozen of North America's altitude research scientists. In planning stages,¹⁶ John wrote,

"The major emphasis of Operation Everest II will be on the changes in the oxygen transport system which make activity possible at levels of hypoxia intolerable without acclimatization".

Sutton's schematic representation¹⁷ of the oxygen transport chain (with simplified labels) is shown in Figure 5. Although Sutton was personally involved in nearly every aspect of OE II, only selected parts of the study related to overall oxygen transport can be detailed here. Sutton's studies on muscle metabolism and function are described by Dr. Green.

Overall O₂ Transport

The first issue was the maximal capacity for overall oxygen transport for subjects at the equivalent of the summit of Mt. Everest. Previous estimates had been made in subjects breathing air and hypoxic gas at altitudes below the summit.^{8,27} These prior estimates and the findings from OE II were in good agreement, and indicated for acclimatized subjects on the summit of Mt. Everest a maximal oxygen uptake of about 1.2 L/min, which is sufficient to support mild exercise. Although the maximum oxygen uptake values on the summit were about 1/4 of those at sea level (Fig. 6), the inter-individual variability observed at sea level was nearly absent on 'the summit'. One possibility was that while circulatory factors played a dominant role in limiting oxygen transport at sea level, pulmonary oxygen transport from ambient air to blood might become progressively more important at altitude.²³

Ventilation: Moving O₂ from Ambient Air to Alveolus

Therefore, one question was whether respiratory muscle fatigue, which might be expected when large volumes of air are ventilated by severely hypoxic persons,

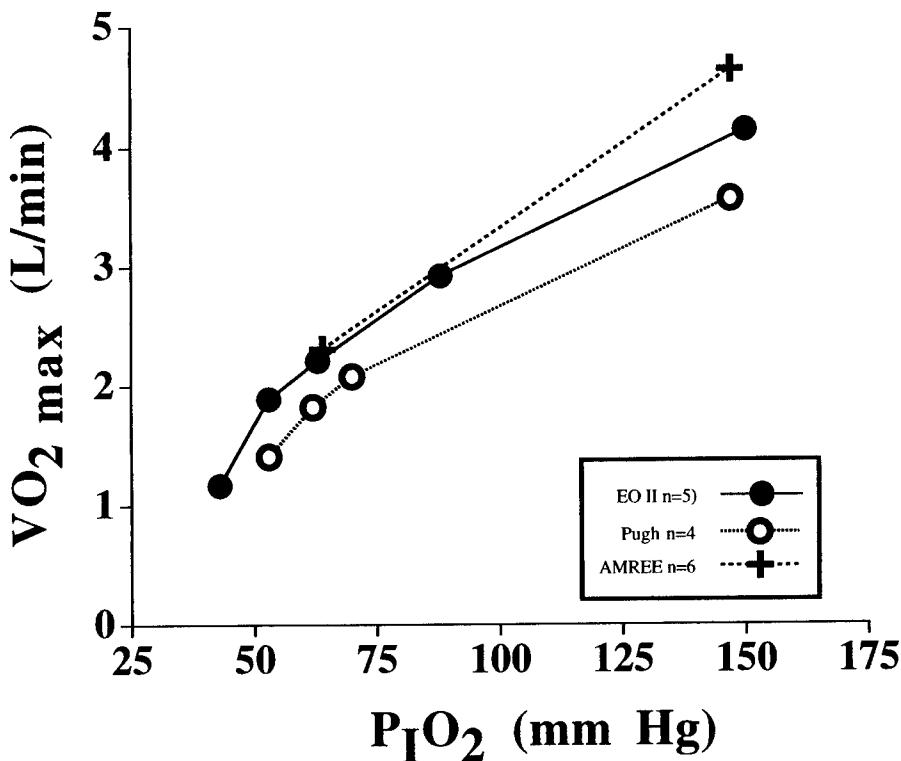


Figure 6 Maximum oxygen uptake in altitude acclimatized healthy men at different inspired oxygen pressures ($P_I O_2$) from sea level to the equivalent of the summit of Mt. Everest. Data are from Pugh et al,⁸ West et al,²⁷ and OE II.¹

limits ventilation at extreme altitude. The measurements during OE II indicated that ventilation increased with power output at all altitudes (Fig. 7A). However with increasing altitude, ventilation for a given power output increased, such that a family of curves resulted, as has been reported by others.^{8,27} The ventilation at maximal effort remained relatively constant from sea level to the equivalent of the summit of Mt. Everest¹ (Fig. 7B) consistent with the idea that in acclimatized subjects, respiratory muscles functioned well. Also, the absence of substantial respiratory fatigue was suggested in that subjects could increase ventilation during exercise sufficiently to maintain the $PaCO_2$ at or below resting levels.¹⁹

However, these findings from OE II differed from those of Pugh et al,⁸ which suggested a fall in maximal ventilation at a very high altitude (Fig. 7B). The measurements of West et al. also predicted a reduced maximal ventilation when acclimatized subjects below the summit of Mt. Everest breathed hypoxic gas to simulate summit $F_I O_2$.²⁷ However, in West's study when the $F_I O_2$ was increased, the subjects were able to increase both maximal oxygen uptake and maximal ventilation. The findings were compatible with better respiratory muscle function during normoxia than during hypoxia, but did not establish whether respiratory muscle fatigue had occurred in the hypoxic subjects. In the actual mountain environment, climbers inspire very cold, dry air, which must be warmed and humidified by the respiratory

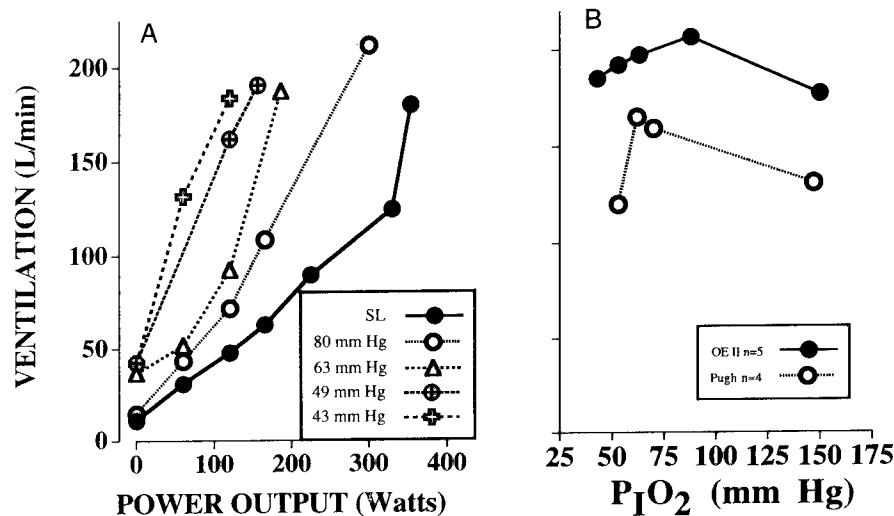


Figure 7A left. Measurements from Operation Everest II¹ showing a family of curves relating ventilation to power output at various altitudes from sea level to the equivalent of the summit of Mt. Everest. **Figure 7B** right. Maximal oxygen uptakes as measured in OE II¹ and by Pugh et al⁸ at different altitudes.

passages. Respiring such large volumes may be painful and irritating to the airways. At the summit of Mt. Everest as simulated in the chamber, the OE II subjects breathed air that was very much warmer, and also had a relative humidity above 80%, which was likely better tolerated than on the mountain. Such factors may have contributed to a better maintained ventilation in the chamber than on the mountain.

Whether or not respiratory muscle fatigue or other factors developed to limit ventilation at extreme altitude, Sutton emphasized the important role played by ventilation in maintaining oxygen transport at high altitude.^{18,19} An illustration involved the examination of the PO_2 gradient from the inspired (P_1O_2) to the alveolar (P_AO_2) air as oxygen uptake increased (Fig. 8A). With increasing oxygen uptake at sea level, ventilation increased such that the P_1O_2 - P_AO_2 gradient stayed nearly constant at ~40 mm Hg. For sea level maximal oxygen uptake of 4L/min, the P_1O_2 was 150 mm Hg. As exercise intensity increased to maximum, ventilation increased enough to maintain P_AO_2 at ~110 mm Hg. The 40 mm Hg PO_2 gradient from inspired to alveolar air reflected the alveolar dilution of the inspired oxygen by CO_2 , where the P_ACO_2 was ~40 mm Hg. At an altitude of ~5000 m, the P_1O_2 is 80 mm Hg, and the maximal oxygen uptake was reduced to ~3 L/min, (Fig. 8A). Hyperventilation had reduced the P_1O_2 - P_AO_2 gradient to about 20 mm Hg. At the equivalent of the summit of Mt. Everest (8848m), where P_1O_2 was only 43 mm Hg and maximal oxygen uptake was ~1.2 L/min, hyperventilation had reduced the P_1O_2 - P_AO_2 gradient to only about 10 mm Hg, (Fig. 8A). Thus for a given oxygen uptake the hyperventilation (which via acclimatization to progressively higher altitudes lowers the P_ACO_2) facilitated oxygen transport by minimizing the PO_2 drop from the inspired to the alveolar air. Sutton visually demonstrated the magnitude of the effect as it varied both with altitude and oxygen uptake (Fig. 8A).

OE II has been criticized for not giving sufficient time during chamber decompression for subjects to become as well acclimatized to altitude, as occurs in moun-

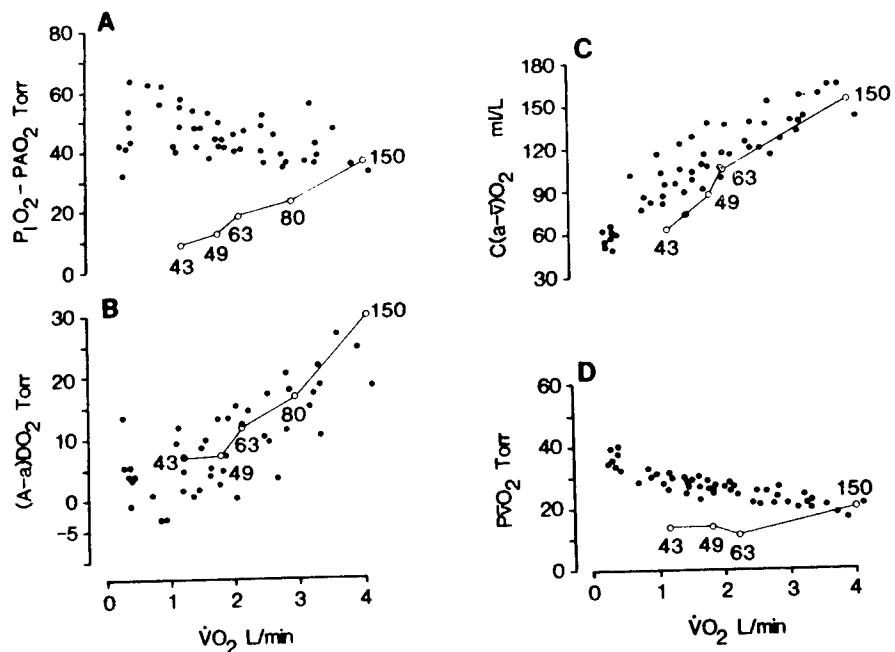


Figure 8 Shown for various oxygen uptakes along the abscissa are the links in the oxygen transport chain. Data are from OE II as reported by Sutton et al.¹⁹ In each panel the filled circles are measurements at rest and during submaximal exercise at sea level. The open circles connected by unbroken lines represent measurements during maximal effort at sea level ($P_1O_2=150$ mm Hg), at 4500 m ($P_1O_2=80$ mm Hg), at 6100 m ($P_1O_2=63$ mm Hg), at 8060 m ($P_1O_2=49$ mm Hg) and at 8848 m ($P_1O_2=43$ mm Hg). Panel A shows the PO_2 gradient from the inspired to alveolar air. Panel B shows the PO_2 gradient from alveolar air to arterial blood. Panel C shows the arterio-venous oxygen content difference. Panel D shows the PO_2 gradient from mixed venous blood to mitochondria (which are presumed to have $PO_2=0$).

tainers. Although the chamber 'ascent profile' in OE II was clearly too rapid for good acclimatization in the early days of the altitude exposure, a slower 'ascent' in the later phase allowed for better acclimatization, as documented by Malconian et al.⁷ (Fig. 9). At issue was whether subjects were or were not well acclimatized at the equivalent of the summit of Mt. Everest.⁷ Clearly, OE II subjects were sufficiently well acclimatized to remain for 8 days and nights at or above 8060 m (PB=282, $P_1O_2=49$ mm Hg), and to achieve oxygen uptakes at 8848 m of 1.18 L/min, all without oxygen. In OE II there were 20 arterial blood samples taken at rest and during exercise in 6 subjects.^{7,19} The resting arterial PCO_2 for 6 subjects on the summit averaged 11.4 and 11.2 mm Hg on two occasions. At the lower end of the PCO_2 range at rest, on 6 occasions (4 subjects) arterial PCO_2 was measured between 9.8 and 8.9 mm Hg.⁷ The lowest value observed was 8.2 mm Hg during exercise in one subject.¹⁹ These values compare with alveolar PCO_2 measurements in one subject²⁶ shortly after climbing to the actual summit (and 10 min. after discontinuing breathing supplemental oxygen) which for 4 measurements ranged between 5.1 and 9.0 mm Hg, (Fig. 9). Variations in PCO_2 within and between subjects, the small number of measurements on the actual mountain, and the differences in the circumstances of the experiments make it difficult to determine whether acclimatization was as good

in the chamber as on the mountain. However in any event, the measurements for some subjects were in rather close agreement.

There was substantial disagreement in the measurement of resting arterial pH (each of 6 subjects on two occasions, mean = 7.56 units) in the chamber compared to that estimated in one subject on the actual summit (7.76 units, derived from alveolar PCO_2 on the summit, and blood HCO_3^- drawn the next day at 8050m). Whether or not such extreme alkalosis occurs in healthy persons at high altitude will have to be determined by future study.

The scientific significance of the ventilatory studies was that there was a curvilinear relationship of PCO_2 to PO_2 for persons acclimatized to altitude, reminiscent of the acute hypoxic ventilatory response determined by carotid body activity. The findings at altitude are compatible with PCO_2 approaching an asymptote at a PO_2 which is between 30 and 37 mm Hg (Fig. 9). Alveolar PCO_2 and alveolar ventilation are closely related. Thus, for a constant PCO_2 production as occurs in resting subjects, alveolar ventilation is inversely proportional to PCO_2 . As PCO_2 gets very small (goes toward zero), ventilation must get very large (approaches infinity). Therefore, one may say that ventilation increases sharply in altitude acclimatized subjects as the PO_2 approaches some limit which may be ~30 mm Hg. A similar ventilatory asymptote at a PO_2 of ~32 mm Hg has been suggested for unacclimatized subjects who are exposed acutely to progressive hypoxia:²⁴

$$V_E = V_o + A/(P_A O_2 - 32).$$

The equation describes an hyperbole relating ventilation (V_E) to $P_A O_2$, where V_o (the horizontal asymptote) is a constant ventilation as $P_A O_2$ becomes very high, and

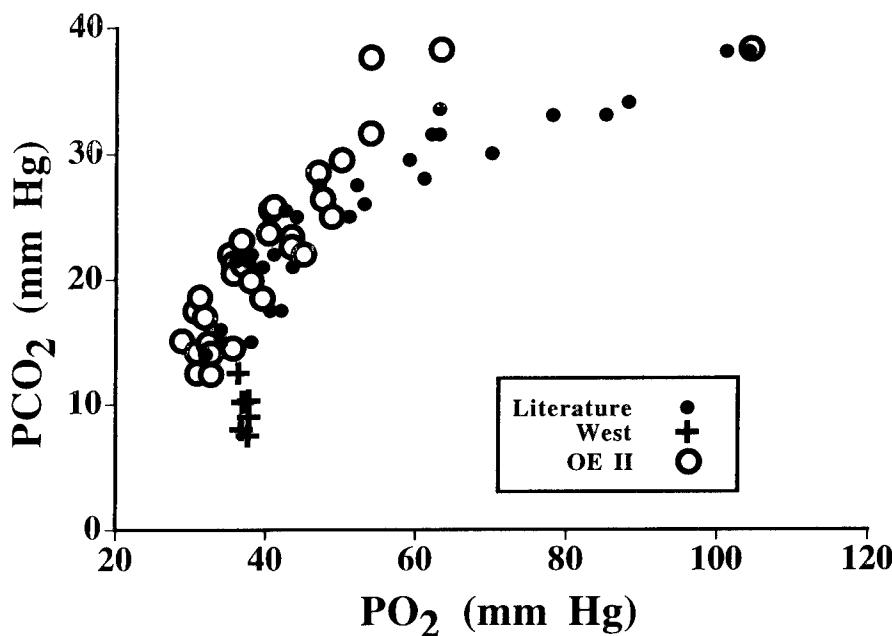


Figure 9 Rahn-Otis diagram showing the curvilinear relationship of PO_2 to PCO_2 for subjects acclimatized to high altitude. Data are redrawn from Malconian et al.⁷

32 (the vertical asymptote) is the PO_2 at which ventilation approaches infinity. The findings from OE II seem to be compatible with the concept that the carotid body exerts important ventilatory control in subjects acclimatized even to the highest terrestrial elevation.

A-a O_2 Gradient: Moving O_2 From Alveolus to Blood

Oxygen moves passively along a PO_2 gradient from alveolus (P_AO_2) to arterialized (P_aO_2) blood. In general, the amount of oxygen which can be transported is the product of the gradient and the pulmonary diffusing capacity, both of which are complex terms. For example, diffusing capacity increases with increasing area of the alveolar capillary membrane, and decreases with increasing thickness of that membrane. It also depends on the rate of reaction of oxygen with hemoglobin, the pulmonary capillary blood volume, and the velocity of red cells through the lung capillary bed. The PO_2 gradient is determined by factors including, diffusion, shunt, and ventilation/perfusion (V/Q) matching. Before OE II, the behavior of the PO_2 gradient was not known at great altitude because arterial blood could not be sampled. Further, the relative roles of diffusion and V/Q were not clear because the specialized measuring techniques were not applicable in the field. Evaluating these factors in extreme chronic hypoxia was important because pulmonary capillary transport has been considered a limiting factor in hypoxia, while pulmonary hypertension and lung edema could degrade both diffusion and V/Q. Sutton's approach was a) to measure the A-a PO_2 gradient during exercises to maximum from sea level to 8848 m, and b) to invite Dr. Peter Wagner's participation for the multiple inert gas assessment of V/Q and diffusion. Groves was enlisted to perform systemic and pulmonary arterial catheterization, which would promote measurement precision.

The A-a PO_2 gradient at sea level, as expected, increased with increasing oxygen uptake (Fig. 8B) ranging from ~5 mm Hg at rest to ~28 mm Hg at maximal effort.¹⁹ With increasing altitude, as the maximal oxygen uptake fell, the A-a PO_2 gradient at maximal effort also decreased. Thus at the various altitudes, as the P_tO_2 fell from 150 mm Hg (sea level) to 43 mm Hg (8848m), the A-a PO_2 gradient was not different from the sea level values, given the amount of oxygen to be transported. These findings at extreme altitude did not exclude the presence of adverse factors which could increase the A-a PO_2 gradient. Indeed, the subjects developed a) pulmonary hypertension with impairment of pulmonary circulatory regulation,³ b) concomitant V/Q mismatch,²¹ and c) evidence of pulmonary edema.²⁵ However, the implication was that these adverse factors did not override the primary determinant of the A-a PO_2 gradient at extreme altitudes, namely the amount of oxygen to be transported.

Circulation: Moving O_2 From Lung To Systemic Capillaries

Sutton stated his hypothesis and his reservations.¹⁹

"We expected that with progressive hypoxemia, a low level of arterial content would be reached where, ... a given amount of oxygen must be transported by an increased cardiac output ... Yet such severe hypoxemia might impair the ability of the heart to provide increased output. At issue was whether, with a progressive decrease in arterial content, circulatory O_2 transport would fail or whether transport would be defended by increased cardiac output."

Cardiac output had been measured using the acetylene method by Pugh et al. on the Silver Hut expedition.⁹ Their measurements at 5800 m had shown that for a given

oxygen uptake, cardiac output was not different from that at sea level. In OE II, cardiac output as measured by three methods simultaneously (direct Fick method with sampling of systemic and pulmonary arterial blood, thermodilution, and multiple inert gas method) showed good agreement between the methods.³ Where possible, the values reported were by the direct Fick method. The OE II measurements showed that at ~6100 m in OE II, the cardiac output value for a given oxygen uptake were not different from that at sea level, and were similar to those previously reported by Pugh et al. (Fig. 10). At the higher altitudes of 8060 and 8848 m, the output values for given oxygen uptakes tended to be slightly higher than at sea level (Fig. 10).

Another way to demonstrate circulatory oxygen transport is to relate the arterio-venous oxygen content difference ($C(a-v)O_2$) to oxygen uptake (Fig. 8C) which provides a useful display of the two components of output as calculated using the direct Fick method. At sea level, $C(a-v)O_2$ widens with increasing exertion to a value approaching 150 ml/L at maximal effort (Fig. 8C). At the highest altitudes of 8060 m ($P_1O_2=49$ mm Hg), and 8488 m ($P_1O_2=43$ mm Hg), the content difference was significantly narrower than for the same oxygen uptake at sea level.

The maintained or higher cardiac output for a given oxygen uptake, a smaller $C(a-v)O_2$ for a given oxygen uptake, a well maintained Starling curve,¹⁰ and maintained cardiac contractility by ultrasound¹² indicated that cardiac function was not seriously impaired with the most severe chronic hypoxia in normal man. The conclusion was that even at the equivalent of the summit of Mt. Everest, circulatory O_2 transport did not fail.

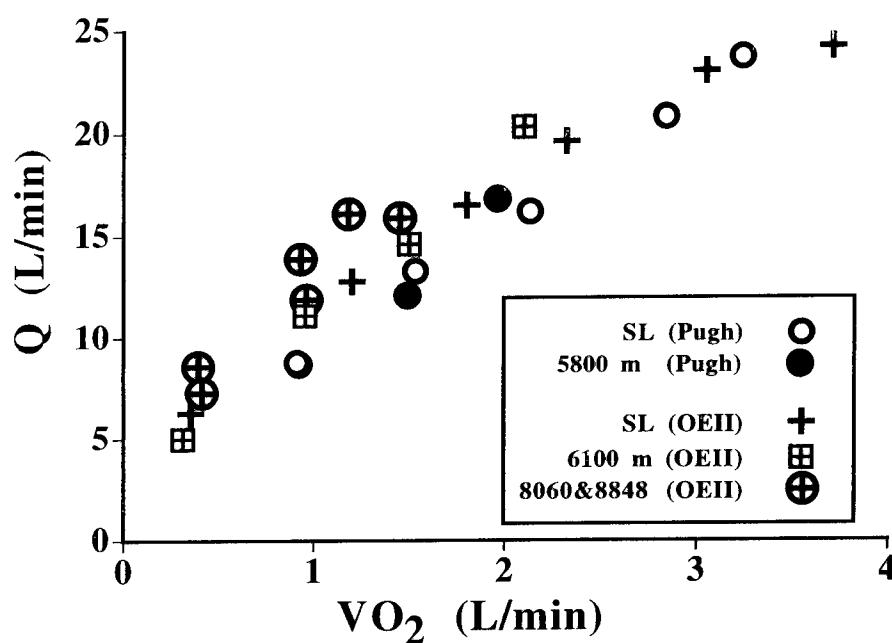


Figure 10 Increasing cardiac output with oxygen uptake at sea level and high altitude. Data are redrawn from Pugh et al⁹ and Sutton et al.¹⁹

An anomaly relative to cardiac output at altitude is that numerous studies have shown for acclimatized subjects at 3000 to 4300 m, cardiac output as related to oxygen uptake is reduced below the values observed at sea level. Yet, cardiac output data from 5800 and 6100 m show no such reduction, and data from higher altitudes allow that outputs at higher altitudes may even be increased above the sea level values. Sutton postulated that arterial and venous oxygen contents and saturations fell so low at extreme altitude that the difference between them must become narrowed if O_2 transport was to be maintained.¹⁹ Data reported in the next section indicate that minimal values for mixed venous saturation and PO_2 are achieved at an altitude of about 6100 m, i.e., well below the summit of Mt. Everest.

The above views have examined only the relation of cardiac output to oxygen uptake. However, an alternative view is concerned with the parallel reduction at altitude of maximal oxygen uptake and cardiac output at maximal effort.¹¹ This view considers that the reduction in maximal oxygen uptake with increasing altitude, may be due in part to limitations in cardiac output. At sea level, a limitation in circulatory function is an important component limiting O_2 transport, and increasing maximal cardiac output is accompanied by increased maximal oxygen uptake.²³ Similar mechanisms could operate at altitude. In such case, if it were possible to increase maximal cardiac output at altitude, maximal oxygen uptake would also increase. Although limitations in pulmonary oxygen transport have been considered to be increasingly important with increasing altitude,²³ the relative roles of the heart and the lung in limiting human performance at altitude need further examination.

Extraction: Moving O_2 From Systemic Capillaries To Mitochondria

In subjects at sea level, oxygen extraction by the systemic tissues increases with increasing exertion, but the mixed venous oxygen saturation usually does not fall below ~20 to 25% (Fig. 11). At altitudes of 6100 m and above oxygen saturations of mixed venous blood during maximal effort fall to ~10%, i.e., half of that observed at sea level (Fig. 11). These minimal values are apparent at 6100 m altitude and do not fall further with increasing altitude. Similar results are apparent from measurements of PO_2 in the mixed venous blood (P_vO_2), (Fig. 8D). Thus at sea level ($P_tO_2=150$ mm Hg) the P_vO_2 falls during maximal exercise to ~20 mm Hg from a resting value of ~40 mm Hg. With maximal exercise at 6100 m ($P_tO_2=63$ mm Hg), the P_vO_2 has a minimal value of ~10 mm Hg, and no further decrease is observed at higher altitudes of 8060 and 8848 m, ($P_tO_2=49$ and 43 mm Hg, respectively) (Fig. 8D).

Sutton commented on several aspects of these novel data which need further study. First, minimal values of P_vO_2 did not occur with maximal exercise at sea level. Sutton indicated that the low arterial O_2 content depressed venous O_2 content, saturation, and PO_2 during exercise.¹⁹ Also, Wagner²² has suggested that as in the lung, the PO_2 diffusion gradient in the tissues increases with increasing oxygen transport. If so, the lower mixed venous PO_2 at great altitude than at sea level was the combined effect of arterial hypoxemia plus the decreased maximum oxygen uptake. Sutton made it clear that the increased extraction at altitude was an important factor maintaining oxygen transport during exercise.

Second, Sutton considered that although the PO_2 in the muscle capillaries was not measured in OE II, it may have been lower than those observed in the mixed venous blood. Supporting evidence was the study of Hartley et al,⁴ who indicated that with

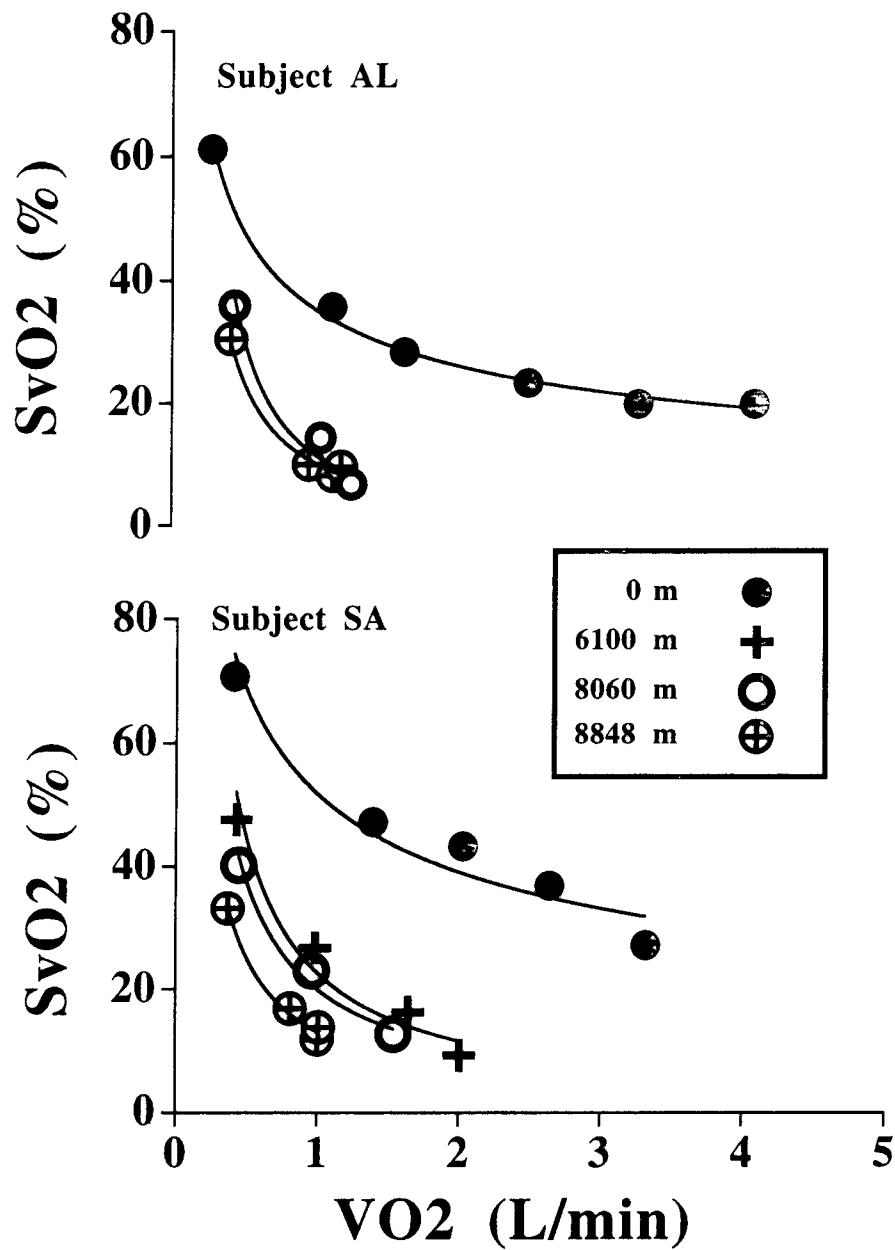


Figure 11 Oxygen saturations in mixed venous blood (SvO_2) with increasing oxygen uptake from rest to maximal effort for two subjects in Operation Everest II at the various altitudes shown. For both subjects values near 10% occurred with maximal effort at altitudes of 6100 m or above.

maximal effort in acute hypoxia, the systemic a-v O_2 difference was 90% of the a-v difference for the exercising leg. Dempsey et al² reported femoral venous PO_2 values as low as 5 mm Hg during exercise in subjects at 3100 m altitude. Thus, under some

circumstances, exercising skeletal muscle is capable of near complete extraction of oxygen from the perfusing blood. Yet, when non exercising tissues become extremely hypoxic, the fraction of cardiac output distributed to working muscle may decrease. Needed are studies which demonstrate the relationship of extraction from exercising, versus non exercising, tissues in humans at very high altitude.

Third, the nadir for the exercise P_vO_2 values occurred at an altitude considerably lower (~6100 m) than the summit of Mt. Everest (8848 m). That is, maximal exercises at altitudes of 8060 and 8848 m resulted in P_vO_2 values that were not different from those observed during maximal effort at 6100 m. Sutton speculated that, "The lower limit for P_vO_2 may be in the range of 10-14 Torr, for these were the minimal values observed at P_fO_2 's of 63, 49, and 43 Torr; and all were less than the lowest value observed at sea level". Of interest, Wagner et al²⁰ showed mixed venous PO_2 values of 13 mm Hg during maximal exercise in subjects exposed acutely to 15,000 feet altitude. Taken together, the data suggest a lower limit of 10 to 14 mm Hg for mixed venous PO_2 .

If so, then in exercising muscle the capillary PO_2 is likely to be less than ~10 mm Hg. Given that the PO_2 in mitochondria is near zero, the pressure gradient from muscle capillary to mitochondria is approximately 10 mm Hg or less during maximal exercise at altitudes of 6100 m or above. Even given this small driving pressure for oxygen, an oxygen transport of slightly more than 1 L/min occurs on the 'summit' of Mt. Everest. Despite the severe hypoxemia, muscle biopsies performed by Sutton and reported below by Green showed remarkable maintenance of muscle high energy stores.

Summary of Altitude Research

The present review of John Sutton's altitude research has focused largely on a single publication on oxygen transport from Operation Everest II, published in the Journal of Applied Physiology in 1988.¹⁹ Such emphasis in no way intends to minimize his major contributions in other altitude research, such as that on Mt. Logan, Pikes Peak, or in Tibet. Rather, this single publication was in a very real sense the *raison d'être* for the whole of Operation Everest II. There were six normal men, who endured the entire experiment from sea level to the 'summit' of Mt. Everest, who had studies from rest to maximal exercise, who underwent 3 separate cardiac catheterizations with measurements at four altitudes including the 'summit', and who submitted to numerous other investigations. All things considered, they provided the most complete descriptions of oxygen transport ever obtained in human beings. The "oxygen transport paper", provided the big picture for all of OE II. It was Sutton's special interest, a joy, and his source of greatest satisfaction. Oxygen transport was the only substantial, primary data set for which he reserved first author privilege. The OE II achievement was a challenge requiring an enormous expenditure of intellectual, emotional, and physical energy. But after all, that was the essence of John Sutton.

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CHAPTER 24

JOHN SUTTON AND SEA LEVEL RESEARCH BRIDGING THE GAP 1941-1996

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Introduction

Like the scientist himself, John Sutton's research cannot be described in pedestrian terms. In the 30 years of his research career he addressed a wide diversity of issues, probed a variety of environments and incorporated a range of participant groups. He employed the tools of basic science to explore mechanisms and applied the knowledge gained from basic science to reduce illness and injury. He addressed the clinical problems of the disadvantaged and diseased, and the performance limitations of the gifted. Throughout he used exercise to uncover mechanisms, to identify failing processes and to alleviate diseases of lifestyle. Nowhere, however, is his passion more evident than in his quest to understand acclimatization both to altitude and to hot humid environments. His altitude research remains as a recurring theme throughout his career and is perhaps the area where he has had the greatest scientific impact. His interest in thermal acclimatization came from accidents resulting from competition in hot, humid environments, an issue that he continued to be actively involved with until his death.

His remarkable productivity can be evidenced not only by his research publications but by the numerous book chapters and articles he wrote addressing clinical concerns especially those induced by the active state.

John was not only a scholar with a passion for application, he was a gifted teacher, enthusiastic and anxious to promote awareness and individual responsibility. He was truly committed to bridging the gap.

Control of Heart Rate

John's formal entry into the scientific community began with an article published in *Lancet* in 1967 while he was at the Garvan Institute of Medical Research. This was a particularly influential study with which to launch a scientific career since it provided evidence that in addition to the adaptation in the extrinsic control of heart rate, mediated by the sympathetic and parasympathetic systems, that occurs with training, the intrinsic heart rate (I.H.R.) is also altered (Sutton et al. 1967). Using both propranolol and atropine to pharmacologically isolate the heart, John and colleagues were able to demonstrate that a lower I.H.R. was associated with a high

aerobic capacity in a large cross section of healthy young males. Moreover, they demonstrated directly that aerobic training significantly reduced resting I.H.R. To examine whether the lower I.H.R. also affects the exercise response, a sample of volunteers also performed treadmill exercise with dual blockage, a somewhat daring manipulation given what was known at the time. Their findings indicated that the increase in I.H.R. with exercise following training was attenuated. The major impact of this work is that the results established rather conclusively that intrinsic alterations in the sinoatrial node must be considered as part of the mechanism governing the bradycardia of exercise training. Interestingly, it was also during this period that John published a clinical article on the hyperkinetic heart syndrome.²⁹ In this study, two individuals with a marked resting tachycardia were described. Both individuals were trained endurance athletes.

It wasn't until early 1970's after coming to Canada that John was able to probe the cellular mechanisms underlying the training-induced reductions in I.H.R. With his first graduate student at McMaster University, Rich Hughson, he examined the potential causes of the reductions in sinoatrial frequency noted with training.¹² Using 60 rats, divided into several groups, a variety of conditions were designed to investigate whether parasympathetic like effects were responsible for the reduction in I.H.R., an hypothesis which they were able to reject. It is perhaps worth noting that in the 20 years following the publication of this study, Dr. Hughson has become an internationally recognized scientist for his work on cardiovascular regulation. To the author's knowledge, rats have never been a preferred species in the numerous studies performed since completion of his doctorate degree.

While at McMaster, John continued to pursue his interest in cardiac physiology both with regard to healthy and diseased populations. In one study,^{32b} published in 1977, differences in intravenous versus oral propranolol administration were used to study I.H.R. because of criticisms that intravenous propranolol may not be as effective particularly during exercise when the sympathetic drive is enhanced. They found that at rest both forms of propranolol administration were as efficient but during exercise the cardiac response was greater after intravenous propranolol. This was an important finding since it indicated that pharmacologic isolation at least for the sympathetic effect was not complete during exercise, and consequently John was able to challenge the interpretation of some earlier work regarding training effects on I.H.R. during exercise.

Two additional studies of note investigated cardiac performance in patients with heart disease and trained athletes. In the study using heart disease patients,⁴³ it was found that an impaired cardiac acceleration occurred during the non-steady state adjustment to exercise. The impairment in cardiac acceleration was also accompanied by a larger time for adjustment in both $\dot{V}CO_2$ and ventilation. It was suggested that these findings may help explain the poor exercise tolerance characteristically noted in patients with coronary disease. The study of athletes (trained swimmers) was used to investigate the effect of apnea and facial immersion on both resting and exercise heart rates and to determine whether the degree of apnea is related to swimming skill or physical conditioning.²² Synchronized swimmers were found to display the greatest bradycardia, an observation that was attributed not to conditioning state but to superior breath hold ability. In one synchronized swimmer, heart rate fell from 74 to 24 b/min at rest, and from 129 to 25 b/min during facial immersion and exercise.

As might be expected, John's continuing interest in the effects of altitude also extended to heart rate control and over the years, he was involved in several studies in this area. The final study, published in conjunction with R. Hughson and others¹⁴ examined the role of the parasympathetic PNS and sympathetic (SNS) systems in the control of heart rate during acclimatization to Pike's Peak, which is some 4300 m above sea level. Power spectral analysis of heart rate variability, a relatively new and unique technique, was used to characterize the PNS and SNS effects. Within the first 4 to 5 days after arrival on Pike's Peak, the elevated resting heart rate observed could be explained by an increased SNS tone and a decreased PNS tone. With a longer period of acclimatization, the PNS indicator returned close to sea level values while the SNS indicator remained elevated. It is perhaps fitting that R. Hughson was John's first graduate student who examined the cellular mechanisms underlying the changes in I.H.R. with physical conditioning in rats.

Exercise and Heat

Nowhere is John's passion and commitment to applied clinical research more evident than in the work that he performed over the years on medical problems associated with mass participation runs and, in particular, exertion-induced heart exhaustion (EIHE). In 1972, while still at the Garvan Institute, St. Vincent Hospital, he published his first major paper documenting the problems encountered in the first Sydney City-to-Surf race, held in 1971.²⁵ Of approximately 2000 entrants, a surprising 29 collapsed during the race and were treated for heatstroke and/or hypoglycemia. Based on this race, a series of recommendations were formulated to address the need for both prevention and for medical treatment of injured participants. In many respects, the City-to-Surf race became a model on how such competitions should be conducted to insure minimal risk to the participants. Careful records were kept of each run, recommendations were constantly upgraded and revised, competitor education, medical and race organization continually improved. As a result, dramatic reductions in the incidence of EIHE were realized.²³ Over a 25 year period (1971-1996), only 3 deaths from heatstroke occurred in approximately 531,000 runners. Throughout John's career, even during his 17 years in Canada, he remained involved in the City-to-Surf race, working closely with Dr. R. Richards who had been appointed as medical coordinator of the run.

John continued to be active in the "heat injury" field throughout his career, lecturing and publishing on a variety of aspects, guided by the belief that "heat injury is a serious, largely preventable and potentially fatal condition."³⁶ As might be expected, he also became intimately involved in the medical problems associated with Canadian "fun runs", previously not allocated much concern given our generally friendly climate. With R. Hughson and others,¹³ it was shown that the safety in Canadian mass participation runs was indeed a serious issue. In 1978, 26 people out of 2900 participants collapsed from heat injury. This study was important in alerting officials to what may be a situation somewhat unique to Canada with the extremes in climate that exist. For competitions conducted in the spring and early summer, there may be an inadequate period for proper acclimatization.

John's final contribution to promoting a safe environment free from EIHE was made just months prior to his death. He organized a major conference in Sydney on Exercise and Thermoregulation which brought together both basic and applied researchers, representing sport and work environments, to address the critical issues.

Fittingly, Dr. R. Richards was honoured for "his contribution to community health and fitness in the safe conduct of community fun runs". In 1980, John concluded in one of his articles addressing the safety issues of mass participation runs that "above all, we must keep the fun in fun runs."³⁶ Many of us remain indebted to John for his unselfish dedication to making such an ideal possible.

Exercise and Endocrine Function

The effect of exercise on endocrine function remained a major interest throughout John's career. His sustained accomplishments in this area were driven both by the curiosity of the basic scientist interested in describing fundamental control mechanisms and the pragmatism of the clinician, intent on alleviating endocrine associated abnormalities. As might be expected, the effect of altitude on hormonal behaviour was never far from his probing intellect. I believe that it is in the area of endocrinology that John's leadership and his competence as a scientist are most in evidence. Few of the major hormones escaped John's experimental interest. Exercise remained the major tool with which to both perturb endocrine function and to examine the role of specific hormones in assisting with the unique challenges of the active state.^{24,38}

Most of John's early work in endocrinology performed while at the Garvan Institute of Medical Research, examined the role of exercise in altering blood hormone concentrations. During this early period, much attention was given to growth hormone, the androgens and cortisol.^{28,30} John also possessed an early fascination with the trained athlete and several of these early studies contrasted the effects of exercise on hormone levels between the untrained, swimmers, runners and rowers.^{30,37} As the nature of the exercise induced alterations in the hormonal response patterns, become clearer, due in large part to the many studies published by John, increasing attention was given to the mechanisms mediating the exercise induced response. As examples, the effects of both alpha and beta blocking agents²⁷ and acid-base alterations²⁶ on growth hormone were examined. This work was also extended to examining the effect of hypoxia.^{31,32a} Indeed, the publication in 1977³¹ examining the effects of exercise and acute hypoxia on growth hormone, cortisol and insulin in addition to several blood metabolites is one of the clearest descriptions of the potent effects of hypoxia on these parameters available at that time.

A series of papers examining the role of the menstrual cycle of the physiologic response to exercise also represented an impressive achievement. These studies conducted by one of John's graduate students and in association with other colleagues, clearly indicated that the phase of the menstrual cycle, follicular versus luteal, is important in modifying the exercise response. Not only was it determined that exercise resulted in elevations in plasma estradiol and progesterone but that the elevations were more marked in the luteal phase than in the follicular phase.¹⁶ In contrast, increases in the follicle-stimulating hormone (FSH) and the catecholamines were more pronounced in the follicular phase.^{16,33} Although no differences in the ventilatory and cardiovascular responses could be detected between the two menstrual phases, blood lactate during heavy exercise was substantially higher during the follicular phase and fatigue was more pronounced.¹⁶ This surprising finding suggests that muscle metabolism and substrate selection may be, in part, dependent on differences in the hormonal response between the two phases. To the author's knowledge, these exciting findings have never been confirmed or extended.

For many years and, indeed up until the time of his death, John remained the resident authority on exercise and endocrine function. Essentially all major reviews on the subject were either written independently by John or in conjunction with others. John also published many reviews on the hormonal changes that occur during altitude acclimatization.

Exercise and Skeletal Muscle

It is perhaps fitting that skeletal muscle should also become of experimental interest in John's incessant quest to understand the role of exercise and altitude on performance. The muscle research was initiated while at McMaster University and continued during his 17 years in Canada. At McMaster, John became a vital member of a team of distinguished scholars in the Department of Medicine, who during the 1970's and 1980's became a dominant international force in the field of exercise physiology. This group composed of such people as M. Campbell, N. Jones, N. Toews and G. Heigenhauser, made an enormous contribution to our understanding of metabolic control, acid-base balance and fluid and ionic regulation during exercise not to mention these innumerable contributions to respiratory and cardiovascular regulation. John's efforts were not only restricted to this group. He collaborated with others in Canada and in other Departments at McMaster University on projects of mutual interest.

Indeed, John's collaborative work with MacDougall, Sale et al.²⁰ resulted in an impressive series of papers characterizing the fibre types in body builders and weightlifters. This work provided the first comprehensive description of skeletal muscle composition and muscle function available at the time and these studies form an essential part of our understanding of the changes that occur with heavy resistance training and immobilization.

With his medical colleagues, the control of the flux rate through the major metabolic pathways and segments became a primary interest. The early work challenged some of the current thinking at that time regarding the control of glycogenolysis, glycolysis and pyruvate oxidation. Earlier studies by this group, as an example, were unable to document an increase in phosphorylase "a" despite the employment of a number of protocols designed to lead to large increases in glycogenolysis.³⁹ These early studies also provided some insight into the role of pyruvate dehydrogenase (PDH) activation on oxidation of pyruvate.³⁹ These investigators were able to show that the activation of PDH can be nearly complete depending on the exercise protocol^{39,40} and that the activation also appears to depend on training state.⁴¹ Even though some of these early results have been modified given improved analytical techniques and sampling schedules, the essential premise of this work remains valid, namely that the production of lactate by muscle may result from factors other than limitations in O₂ availability. The imbalance between the rate of glycogenolysis and glycolysis controlled by phosphorylase and phosphofructokinase and the rate of pyruvate incorporation into the Kreb's cycle controlled by PDH must also be recognized. This work continues today at McMaster led by George Heigenhauser, one of John's former colleagues and by Larry Spriet, an ex-student in the Department of Medicine.

In addition to these studies, John was also intimately involved in research examining metabolic control in working muscle. These investigations addressed the role of the purine metabolism on uric acid formation³⁴ and the role of induced changes in acidosis and alkalosis.³⁵

Indeed a series of impressive studies was published examining muscle metabolic control using a protocol of extreme, intermittent cycle exercise designed to result in maximal stimulation of glycolytic flux rates.^{15,21} Among other things, these investigators were able to demonstrate profound changes in phosphorylation state with this type of exercise and the relationship between phosphorylation state, glycolytic flux and oxidative phosphorylation. This exercise protocol was also used to examine acid-base control and the role of strong ions on the changes in hydrogen and bicarbonate that occur¹⁷ as well as the effect of acetazolamide, an inhibitor of carbonic anhydrase.¹⁸ During these studies, the investigators also examined the viability of the Stewart approach for understanding acid-base balance based on physicochemical principles.

Perhaps as important as the research findings that the McMaster group has been able to generate is the legacy left by the graduate students. Many of these graduates have continued with the muscle metabolic studies initiated at McMaster and, in the process have made significant inroads and developed impressive scientific reputations.

Among the numerous research projects that John and I have collaborated in over a period of 20 years, none were more satisfying than the altitude acclimatization projects. My involvement in altitude research began with the invitation by John to participate in studies examining the skeletal muscle adaptations during Operation Everest II. Operation Everest II was an extremely ambitious project, conceived and organized by C. Houston, A. Cymerman, and J. Sutton, and involving numerous scientists, designed to examine the wide spectrum of changes that occur during acclimatization to altitude. To minimize the effect of the many additional stressors experienced during actual mountaineering, the study was conducted in a hypobaric chamber, gradually decompressed over a 40 day period to a simulated altitude equivalent to Mount Everest.¹¹

The muscular component focused on two issues, namely the nature of the adaptations that occur and the significance of the adaptations in muscle energetics during maximal voluntary cycle exercise. As expected, acclimatization resulted in a progressive blunting of the metabolic response observed in the vastus lateralis muscle at exhaustion.⁴ Muscle energy potential, defined by the concentration of the high-energy phosphates, remained more conserved with progressive hypobaric hypoxia and muscle lactate was substantially reduced. These changes could not be explained by a depletion of the muscle substrate, glycogen⁴ or on the changes that occurred in the enzymatic pathways of energy supply.⁵ Muscle glycogenolytic and glycogenolytic potentials, as measured by the maximal activities of a number of enzymes, were protected despite substantial losses in muscle fibre area. Mitochondrial potential was depressed but not until the final week of extreme simulated altitude. Although, muscle fibre capillary number remained unaltered, the reduction in fibre area promoted an increased capillary to fibre area potential and conceivably an improved perfusion potential.

The mechanisms underlying the reduction in muscle lactate at maximal exercise, previously observed in blood following acclimatization and labelled the lactate paradox⁹ remain an enigma. The fact that the effects were in large part reversed soon after the return to normoxia suggests an inhibiting effect of the nervous system on muscle activation occurred, however a failure in one or more of the excitation-contraction processes within the muscle cannot be excluded.⁴ Dill was one of the first to observe the lactate paradox and suggested that "It is as though the body, realizing the

delicacy of the situation with regard to oxygen supply, sets up an automatic control over work which renders impossible the severe acid-base disturbances which can be voluntarily induced at sea level.¹² Interestingly, a recent report has documented the existence of the lactate paradox in patients with chronic heart disease.¹ If Dill is correct, it would appear that even in pathological states protective adaptations may occur during exercise.

In an additional collaborative venture examining altitude acclimatization, metabolic control in skeletal muscle during exercise was examined in conjunction with central circulatory changes⁴² following 21 days residency at Pike's Peak (4,300m). At a given submaximal absolute power output, acclimatization resulted in less of an imbalance between ATP production and ATP utilization, resulting in more protected high-energy phosphate state. Moreover, acclimatization also resulted in a reduction in muscle lactate that could be explained by a depression in glycolysis.⁷ Interestingly, these changes were not accompanied by increases in mitochondrial capacity, increases in capillary to fibre area ratio,⁷ or by changes in whole body exercise $\dot{V}O_2$.⁴² However, leg $\dot{V}O_2$ appeared to be depressed on acute exposure to altitude,⁴² which could explain the metabolic alterations that occurred. It is possible that the reduction in plasma volume induced on arrival at altitude might be implicated in the responses that were observed. In general, the acclimatization responses in muscle metabolism remain intriguing, since they appear to occur in the absence of increases in mitochondrial potential which has previously been hypothesized to be essential for such adaptations at least during sea level training.¹⁰ However, even at sea level, extensive adaptations appear to occur soon after the onset of training and in the absence of an increase in mitochondrial potential.⁸ John was a co-investigator in some of these short-term training studies.^{6,8}

John's abrupt and unexpected death obviously truncated a research career that would have continued to be characterized by a remarkable productivity. No doubt the major themes of his work, endocrinology, temperature regulation, muscle metabolism, and, in particular, altitude acclimatization would have remained his dominant interests. However, would a personality of such energy and enthusiasm have been satisfied to remain within these limits? It appears not. On February 5, 1996, two days before his death, John wrote to me, raising the question "Are you interested in being a scientist associated with Spirit of Australia - South Pole Expedition?" I suspect that my reaction would have been positive and that our satisfying collaboration which had extended for some 20 years would have continued.

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CHAPTER 25

JOHN SUTTON ON MOUNT LOGAN

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John Sutton arrived at the Logan base laboratory at 16:30 on Sunday July 22, 1973 - unexpected, uninvited, and unwanted.

Fortunately he overcame my protests and for the next five years was a major player in the High Altitude Physiology Study, called HAPS or simply the Logan project. John was the principal author of nine of the 28 medical papers from the Logan project to be published in peer-reviewed journals.⁵

HAPS began tentatively in 1967 with US Army support for one year to study the implications of high altitude for military operations. This was stimulated largely by the number of casualties from high altitude illness experienced by the Indian Army during the Sino-Indian conflict in 1962-1963.

The plan was deceptively simple. From a well established base at 2500 feet on Kluane Lake in the Yukon, subjects and scientists and instruments would be flown 90 miles to a facility erected at 17,500 feet on the great plateau beneath the summit of Mount Logan. This facility, affectionately (if misleadingly) called Logan High, would be supplied with fuel and food and living quarters to accommodate safely, and with reasonable comfort, up to 15 persons. The first building was of pre-fabricated plywood, flown up in the spring of 1967.

After three seasons it became obvious that the abrupt transition from 2500 to 17,500 feet could cause serious altitude illnesses, as we should have expected. Thereafter a support team of 8-10 men and women, and some of the scientists were flown to an intermediate camp at 10,000 feet and from there climbed to Logan High in 10-12 days, which gave them time for acclimatization. However, we had to fly some of the scientists directly up from Base, anticipating some altitude illness for a few days after arrival.

The safety and success of HAPS depended on a small STOL aircraft, a superb pilot, and on weather and snow and wind conditions. In ten years more than 250 flights were made: twice aircraft made emergency landings but were only temporarily out of commission. Several times the plane was stuck in deep snow at Logan High and required strenuous work and several hours to free. A third of planned flights were aborted due to weather.

No one was injured or seriously ill at Logan High. Two daring subjects damaged their knees sliding on cardboard from the peaks above Logan High. Two scientists and eight subjects were evacuated because of one type of illness or another. The worst injury happened at Kluane Base, when John Sutton nearly drowned and injured his knee quite badly, trying to cross a small stream!

The Arctic Institute of North America (AINA) was home to HAPS throughout its life, providing the staff and infrastructure at Kluane Lake, the aircraft and pilot—and respectability to a project that at first seemed slightly ridiculous to serious scientists.

Funding had to come from other sources: the Fleischman Foundation, the Defense Research Board (Canada), and the Kresge Foundation. After 1970 these resources dried up, and annual grants of \$50-75,000 were awarded to HAPS by the National Institutes of Health for the next eight years.

From the beginning, the HAPS objectives were:

“To dissect the various responses to hypoxia which occur in water and electrolyte distribution, in hormone release, in the ventilatory and circulatory systems, and in the brain (as evidenced by the electroencephalogram) in order to understand more clearly the patho-genesis of altitude illnesses and the evolution of acclimatization.”⁵

Throughout the ten year project we believed that:

“... a better understanding of the changes which occur in healthy persons at high altitude will contribute significantly to better understanding of the changes which occur in patients with hypoxia due to illness at low elevations.”

After the US Army discontinued support in 1968, the Canadian Armed Forces provided a wealth of supplies, equipment, and transport, for the rest of the project. Canadian elite troops were “volunteer” subjects for a few years. After this we recruited non-military subjects, mostly young mountaineers attracted by six weeks on a high mountain.

As word spread we had many applicants, and choosing the best became an interesting and demanding task. Six references and a personal interview produced very compatible groups of ten to sixteen men and women each year. They were given travel expenses from home to Kluane Lake Base camp, a small salary, and room and board for six weeks. HAPS appealed to the adventuresome though less to serious scientists at first: working and living conditions at Logan High were sparse and many doubted that any meaningful data could be obtained. After two fumbling years, some excellent work was done.

To John goes most of the credit for attracting good scientists and developing protocols for the various studies. In the final years John also arranged to do baseline studies on our subjects at McMaster University, after which his department chairman said, only half in jest, “John not only borrowed most of the equipment but also some of our key staff—on the basis that I had told him he could have a ‘few’ needed items”. At Logan High John was hyperactive, leading good studies despite what must be called primitive conditions.

John and I wrote the annual renewal request to NIH, and were solicitously treated by Dr Claude Lefant and Dr Sue Hurd despite their being stranded at the 9000 foot camp for several days of storm during their site visit.

Accomplishments

In 1970 the HAPS team published the first description of high altitude retinal hemorrhages (HARH) which were seen in 9 of 25 persons taken rapidly or climbing slowly to 17,500 feet.^{1,2,8} HARH were not associated with symptoms of altitude illness, or medication. Further studies by HAPS,^{2,8,12} reported HARH in 22 of 49 subjects and noted a positive association with exertion. Fluorescein angiography showed

capillary leakage, and fresh hemorrhage after maximal exercise. These reports stimulated others to many observations and studies over the next twenty years.

John led studies of sleep hypoxemia due to periodic breathing which was present in everyone after arrival at 17,500 feet and was unchanged by a month of acclimatization there. This work showed that acetazolamide eliminated nocturnal periodic breathing and consequently decreased arterial desaturation during sleep.^{9,10,14} Subsequent studies from HAPS confirmed the initial impression that symptoms of AMS were decreased by acetazolamide and stimulated its widespread use for prevention.^{15,16}

HAPS led John to studies of pulmonary ventilation and his data showed for the first time that the worse was the ventilation, the more severe the symptoms of AMS would be.⁷ He believed, ahead of his time, that:

"...AMS is caused by cerebral edema and is not only associated with the severity of hypoxemia ... but with pulmonary gas exchange abnormalities ... leading to a vicious cycle of worsening hypoxia and worsening AMS."¹⁰

Early in the project we fortuitously noticed that those coming down after a stay at Logan High showed wide-based gait ataxia. At first we attributed this to the clumsy Mickey Mouse boots, but soon identified this as an early sign of cerebral edema. This unpublished observation has since been explored by others and is an important warning of HACE.

Recognizing a unique opportunity, in 1975 John began a series of teaching seminars for our support group and scientists, focused on respiratory and circulatory physiology which led to discussions of altitude illness and acclimatization. Here John was in his element. The informal classes were enthusiastically received, even at Logan High and became an important part of the program.

Looking back 25 years it is gratifying that the one hundred and twenty men and women worked so compatibly with 15-20 scientists; the success of HAPS was due largely to the people involved. Over the years about a third of the subjects have gone on to health related careers, and John was undoubtedly instrumental in their choices.

After HAPS, John joined me in my Arctic Institute Mountain Medicine conferences which he and I soon expanded into the Hypoxia Symposia of today. HAPS also led to Operation Everest II in 1985,⁶ and to John's brilliant achievements in exercise physiology (described elsewhere in this book).

John Sutton was everything and everywhere; he and I formed a partnership which endured until his death. Our talents and faults meshed wonderfully well and he was like a younger brother to me for more than twenty years.

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CHAPTER 26

HYPOXIA IN EVERDAY LIFE OVERVIEW

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Considering that we humans can neither store nor make oxygen in our bodies it is not surprising that hypoxia is common in every day life. Fortunately our oxygen transport and utilization system makes mild hypoxia almost unnoticeable. But many environmental, respiratory, circulatory and tissue conditions do cause clinically important signs and symptoms and even death.

Table 1
Types of Hypoxia

ENVIRONMENTAL	CIRCULATORY
High Altitude	Heart Failure
Air Pollution	Anemia
VENTILATORY	HISTOTOXIC
Airway Obstruction	Cyanide Poisoning
Alveolar Defect	Other Cell Poison

I am often asked why studies at high altitude concern anyone except climbers and other mountain visitors. I answer that altitude research sheds light on many common causes of oxygen lack among low altitude dwellers. In this chapter I look briefly at a few of the many causes of oxygen lack to which we are or may be exposed in daily life.

Table 2
“STRUGGLE RESPONSES” to Hypoxia

Increased Breathing
Increased Heart Rate and Output
Increased Circulating Red Blood Cells
Decreased Blood Flow to Non-Essential Parts
Enzymatic Changes Permitting Some Anaerobic Work

Table 3
Examples

	PaO₂	PaCO₂	Hgb
Normal			
SL	88	42	15
3100m	56	33	16
6100m	41	21	16
7600m	37	13	18
Pink Puffer	70	37	15
Blue Bloater	50	51	17
CMS	47	34	20
Congenital	52	37	
Cardiacs	(33-75)	(33-47)	

For example, by far the most frequent cause of everyday hypoxia is flying because aircraft cabin altitude is maintained at 5,000 to 7,500 feet, often for many hours, depending to some extent on the actual altitude above the earth. Hypoxia in aviation is discussed later in this book.

Environmental Hypoxia:

Altitude

Hypoxia due to thin mountain air has been studied for more than a century, and for twenty years has been a theme of these Symposia. But the more we have learned, the more there is to examine with our ever-changing technology.

The tremendous increase in mountain tourism and climbing in the last thirty years has made altitude a common cause of hypoxia today. Others have discussed the effects of high altitude in the proceedings of previous Hypoxia symposia, and I will mention them only briefly.

Among many responses, some but not all causes of hypoxia stimulate an increase in pulmonary artery pressure roughly proportional to the duration and severity of low oxygen partial pressure in the lungs. Alveolar hypoxia releases biologically active substances which affect pulmonary vessels and provoke the spectrum of altitude illnesses and the evolution of acclimatization. Worthy of note is the fact that tissue hypoxia which results from other causes, in which alveolar oxygen partial pressure is normal, do not cause pulmonary hypertension.

Polluted Air

Among the many other causes of environmental hypoxia are carbon monoxide, propane, carbon dioxide, and nitrogen. Many other gases are toxic but cause hypoxia secondarily due to alveolar damage resulting in interstitial and/or alveolar edema.

Each year 1,500 persons die from carbon monoxide and 10,000 more require medical care in the USA. Incomplete combustion of any substance can produce CO including burning of fossil and synthetic fuels by automobiles, space heaters, and specially in confined places like parking garages, skating rinks, fishing shanties, tightly woven tents and many more. Carbon monoxide is non-toxic but kills from hypoxia by displacing oxygen in hemoglobin. CO also compromises muscular activity, including the heart, because it combines with myoglobin as strongly as it does

with hemoglobin. This is discussed elsewhere in this book. CO can be more accurately described as a circulatory cause of hypoxia.

More than half of atmospheric CO is formed from oxidation of methane. This is produced in huge volumes from cattle feed lots, rice paddies, termite nests and rotting biomass such as marshes. Methane is inert and kills by displacing air in the lungs. Some unwary people have died from methane inhalation after falling into manure pits! Methane in the atmosphere is twenty times as effective as CO₂ in trapping infrared light and thus increasing the greenhouse effect, even though there is less than half as much methane as CO₂ in the atmosphere.

Like methane, carbon dioxide kills by suffocation, displacing air in the lungs. A sensational epidemic occurred in 1987 when a tide of CO₂, released suddenly from Lake Nyos in Cameroon flowed down a valley and suffocated 1,700 people and thousands of cattle. Smaller episodes have occurred near other African lakes. Occasional deaths have been caused by pools of CO₂ in wells or in desert 'sinks'.

Around 1620 Johann Baptiste Van Helmont made a new gas by dripping acid on limestone, and showed that it was the lethal agent in a celebrated cave in Italy called La Grotte de Cane. Heavier than air, CO₂ pooled on the floor of the cave, instantly killing dogs that entered, but not affecting taller humans.

Propane and natural gas contain carbon monoxide, and also cause suffocation (asphyxia). Liquid nitrogen used for instant freezing of many materials, has been fatal when used in inadequately ventilated space. Gaseous nitrogen, used commercially in containers where all air must be excluded, has also killed careless workers. Like nitrogen, the evaporation of solid carbon dioxide (dry ice) has also been fatal for workers handling it in a poorly ventilated area.

Silo filler's disease causes hypoxia by the corrosive effect of nitric acid formed in fermented silage; it is rare thanks to education. Many other vapors cause hypoxia by their direct effect on lungs. Gas warfare in WWI killed or permanently disabled thousands; other toxic gases have occasionally been used against people since then. In 1984 many thousands died and many more were lastingly injured when a pesticide factory in Bhopal, India exploded releasing methyl cyanide which caused pulmonary edema, killing by respiratory hypoxia.

Table 4
Responses

High Altitude		Airway Obstruction	
Ventilation	Increased	Ventilation	Obstructed
Cardiac Output	Increased	Cardiac Output	Increased
Pulse Rate	Increased	Pulse Rate	Increased
Hemoglobin	Increased	Hemoglobin	Unchanged
Cell Activity	Altered	Cell Activity	Unchanged

Respiratory Causes

This category includes any illness or damage that impedes the flow of ambient air through the airways into alveoli and diffusion of oxygen across the alveolar walls into blood. Examples are: dysfunction of the respiratory control mechanism, airway obstruction, paralysis of respiratory muscles, or interstitial or alveolar pathology among others.

Sleep apnea

Control of respiration has fail-safe features in several locations. Whether central or peripheral, sleep apnea causes brief but repeated periods of mild arterial oxygen desaturation. Over time these cause pulmonary hypertension and may result in right heart failure. Misnamed Ondine's Curse and often called Pickwickian syndrome (a literary misnomer), advanced sleep apnea victims have some of the features of Monge's disease (CMS or Chronic Mountain Sickness). Sleep apnea is discussed in another chapter in this book.

Table 5
Chronic Mountain Sickness
Subacute Mountain Sickness

Polycythemia			
Cyanosis	Dyspnea		
	Blunted HVR		
	Right Heart Failure		

SIDS

It is claimed that more very young children die from Sudden Infant Death Syndrome (SIDS or Cot Death) every year than all who die from cancer, heart disease, pneumonia, child abuse, AIDS, and cystic fibrosis combined.

Table 6
Sudden Infant Death Syndrome Crib Death

Sleep Apnea	Arrhythmia	
	Blunted HVR	

SIDS is a form of sleep apnea which affects only infants and young children, causing respiratory arrest, often after several premonitory "near misses". Whether due to sleeping position, asphyxia from bedding, central respiratory failure, or some external cause is unknown today. It is a tragic and important type of hypoxia in everyday life. Survivors of many "near misses" have been brain damaged by these repeated episodes of apnea.

Cystic fibrosis

This recessive genetic defect is manifest by accumulation of excessive thick mucous in lungs which are normal at birth. Repeated accumulation of this viscous material in small airways causes hypoxia and slowly leads to membrane thickening, pulmonary hypertension and recurrent infection.

Chronic Obstructive Pulmonary Disease (COPD)

COPD is the most common lung disorder and limits activity by impeding the flow of oxygen into blood. Although 'essential' or cystic emphysema occurs, the term usually refers to chronic bronchitis and emphysema. About 16 million people in the United States have COPD, and each year 90,000 die from associated pneumonia, respiratory failure, or other complications such as right heart failure due to pulmonary hypertension. The incidence of COPD is said to be increasing by more than 5% annually.

Many individuals with COPD have arterial oxygen levels equivalent to those experienced at 12-15,000 feet but are still able to engage in near normal activities. Some aspects of their tolerance resemble the acclimatization developed by healthy altitude residents and long term visitors.

Table 7

Emphysema - "Pink Puffer"		Emphysema - "Blue Bloater"	
Ventilation	Increased	Ventilation	Depressed
Cardiac Output	Normal	Cardiac Output	Increased
Pulse Rate	Normal	Pulse Rate	Increased
Hemoglobin	Normal	Hemoglobin	Increased
Cell Activity	Unchanged	Cell Activity	Unchanged

Asthma impedes airflow by bronchial and bronchiolar spasm. It is common, usually short-lived though recurrent, but rarely may cause death due to respiratory hypoxia.

Drowning

The human brain is irreparably damaged after six or at most ten minutes of anoxia. How then have a number of children and adults survived, undamaged, after complete submersion for an hour?

Table 8

Dive "REFLEX" or "RESPONSE"	
SHORT PLANNED DIVE	LONG OR UNKNOWN DIVE
Initially Heart Rate Slows	Heart Rate Slows
Heart Rate Increases as Needed	Heart Rate Remains Slow
Muscles Receive Blood on Demand	Little or No Blood to Muscles
Acidosis is Slight	Acidosis Becomes Severe

Diving mammals often stay submerged for an hour during which they can see, think and swim actively without breathing. This is attributed to the dive response which immediately halts breathing when a dive is started, and soon diverts blood away from non-essential to vital organs. Conflict between the need to conserve oxygen and the demands by swimming muscles is resolved for a short period by changing metabolic fuels and "going anaerobic". Oxygen is also "borrowed" from saturated myoglobin, which acts as a kind of storehouse.

Humans are different. Long submersion is only survivable in water which is near freezing temperature. Central reflexes immediately halt breathing. During the first ten minutes of accidental submersion, the diving response protects the brain by redirecting oxygenated blood to it and to the heart and a few similarly essential sites, and by shutting off blood to skin and extremities and soon to kidneys and liver. The heart slows markedly (reducing its demand for oxygen) and blood flow to the brain soon decreases. Total body metabolism decreases.

After ten minutes in freezing water, hypothermia due to passive heat loss further slows the demand for oxygen, and decreases metabolism; the victim becomes unconscious. Children lose heat faster than adults due to their greater ratio of surface area to body mass. Thus the first defense is the dive response, the second and final is hypothermia. However studies suggest that passive cooling may not be rapid

enough to absolutely protect the brain; other currently unknown factors may be operative. Not many such victims are fortunate enough to survive but if so, most are not brain damaged.

A swimmer may hyperventilate before a sprint, or for a long underwater swim, hoping to increase blood oxygen content; subsequent breath holding has caused death from acute hypoxia when the hypoxic ventilatory response failed before the hypercapnic response was activated. Death after a long deep breathhold dive is due in part to the rapid diffusion from pulmonary capillaries into alveoli resulting from the rapid expansion of the lungs while surfacing too rapidly.

Circulatory Hypoxia

Any interference with the carriage or delivery of oxygen can be described as circulatory hypoxia. This includes anemia, hypovolemia (shock), abnormal hemoglobin, circulatory failure (cardiac or peripheral), congenital heart or vascular disease.

Carbon monoxide binds with hemoglobin 250 times more strongly than does oxygen which it displaces. CO is not toxic: death is due to hypoxia because a concentration of only 0.1 percent CO in air will bind with half of the hemoglobin and be fatal. As mentioned above, CO also binds with myoglobin and may cause problems.

Certain toxic chemicals combine with hemoglobin to form methemoglobin which does not carry oxygen and thus causes hypoxia

Severe anemia, and hemorrhage decrease oxygen carrying capacity and therefore content and cause hypoxia. If chronic, some of the responses to anemia resemble those due to high altitude; if acute, the "struggle responses" appear. Lack of circulating hemoglobin is not an uncommon cause of everyday hypoxia. Interestingly, like CO poisoning it does not cause pulmonary hypertension.

A young woman bled internally from a ruptured ectopic pregnancy. She had a cardiac arrest in the ambulance and was comatose and in shock, with a hematocrit of 13% in hospital. She remained comatose for a month then began slow recovery. This is a severe case of circulatory hypoxia.

Mutant hemoglobins, including sickle cell factors cause hypoxia, which is exacerbated by exercise or travel to altitude. Adverse reactions are primarily from multiple thrombo-embolism resulting from clumping of the deformed sickle cells which is reversed by oxygen.

Tissue (Cellular) hypoxia

A variety of enzymatic poisons which interfere with the mitochondrial energy cycles cause tissue or cellular hypoxia. The role of molecular dynamics in tissue hypoxia is being studied extensively, and discussed in other chapters of this and previous Hypoxia Symposium books.

Excessive oxygen demand, due for example to strenuous exertion, high fever, hyperthyroidism, hypovolemia, anemia - or anything which increases tissue demand without increasing oxygen supply may result in hypoxia severe enough to cause signs or symptoms. The delirium of a thyroid storm may be exacerbated by cerebral hypoxia.

On the other hand, hypothermia and hypoglycemia interfere with the higher brain functions and cause problems which are similar to, and synergistic with those of hypoxia.

Summary

From this brief over-view it is obvious that many influences can and often do cause lack of oxygen. At some point the decreased oxygen partial pressure, however and wherever it originates may cause significant signs and symptoms; this varies considerably among individuals. A few causes of hypoxia, perhaps more than we currently recognize, stimulate extensive physiological changes which, in reference to high altitude hypoxia, we call "acclimatization".

CHAPTER 27

HYPOXIA AND AIR TRAVEL

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Introduction

At the present time approximately one billion people throughout the world travel on commercial aircraft each year.¹ In the United States alone, more than 80% of adults have flown at least once and almost 500 million passengers were reported to have traveled on commercial aircraft in 1992.³² These passengers include individuals of all ages, from infants to the elderly, as well as individuals with a wide variety of underlying medical conditions. For the vast majority of individuals commercial air travel is uneventful. Some passengers with underlying illnesses may, however, experience significant physiological stress and exacerbations of their ailments. This is especially true of elderly patients with cardiopulmonary disease.^{8,10,38,47,48,49,74,100,109} In order for physicians to properly evaluate and advise such patients prior to air travel, they must have a basic understanding of the physiological stresses of commercial air travel, the effect of these stresses on medical disorders that are susceptible to in-flight exacerbation and knowledge of specific guidelines for the management of these disorders.

Epidemiology of In-flight Medical Problems

The exact incidence of flight-related illness or deaths is unknown, since no systematic method of reporting and recording such information exists. There are, however, several studies in the medical literature which provide some insight into the magnitude of this problem.

From 1986 to 1988 the Federal Aviation Administration mandated that all U.S. commercial airlines report every case in which the on-board medical kit was used in flight. It is estimated that 900 million passengers flew on U.S. commercial aircraft during these two years. A report published in 1991 revealed that there were 2,322 medical emergencies and 33 in-flight deaths during this period. The on-board medical kit was used 2,293 times in response to these emergencies, with the kit used by a physician in more than 85% of these cases. The most common presenting complaints were pain (12%), with chest pain the complaint in 205 of the 280 pain reports, unconsciousness (10%), nausea and/or vomiting (7%), shortness of breath (6%), and problems related to various myocardial disorders (4%) (Fig. 1). Of the 33 reported deaths the cause was unknown in 11 cases. Among the other 22 deaths, 16 were related to cardiac disorders, 2 were due to accidental causes, 2 were caused by terminal cancer, 1 was related to an allergic disorder and 1 was attributed to AIDS (Fig. 2).⁶¹

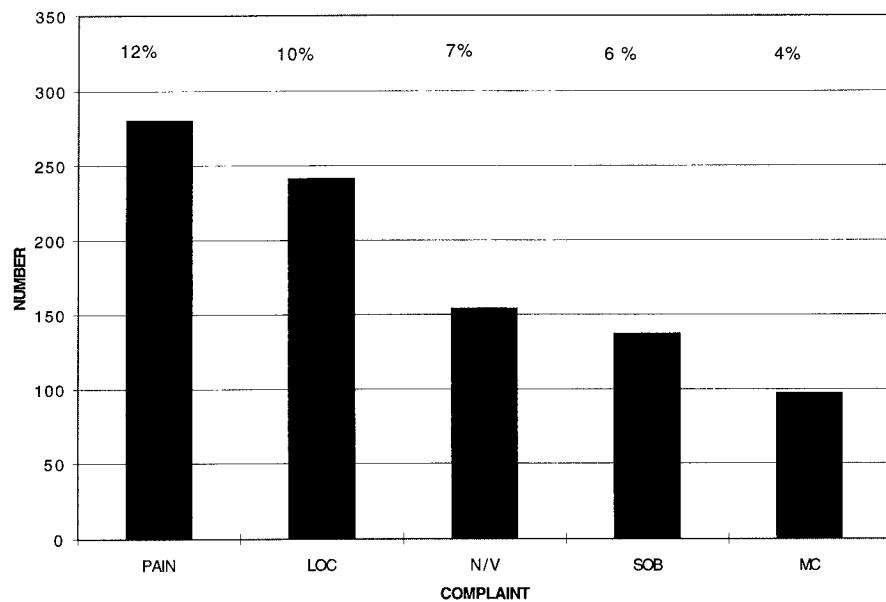
MOST COMMON COMPLAINTS (N=2,322)

Figure 1 Most common presenting complaints of in-flight emergencies during commercial air travel; pain, loss of consciousness, nausea/vomiting, shortness of breath, myocardial. From Hordinsky and George⁶¹.

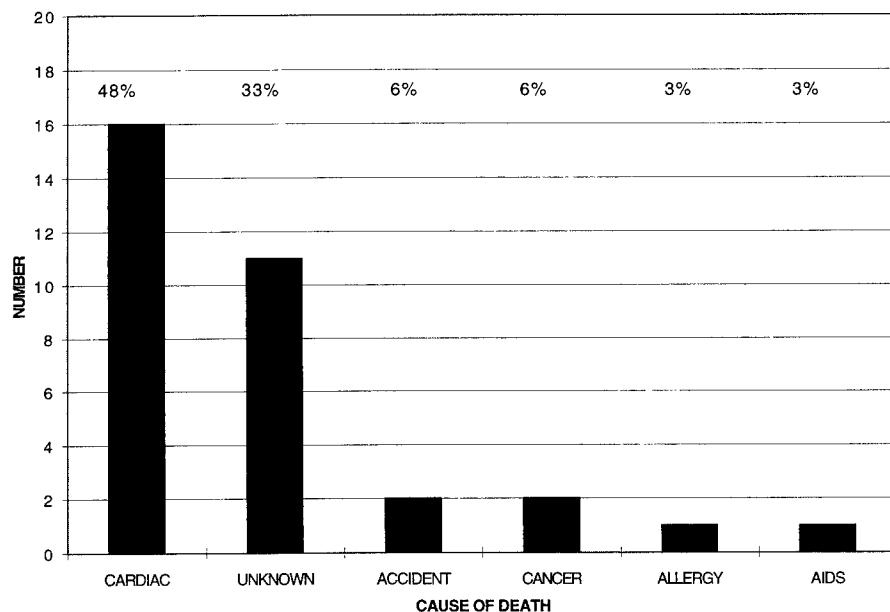
IN-FLIGHT DEATHS (N=33)

Figure 2 Causes of deaths during commercial air travel. From Hordinsky and George⁶².

A related report, also published in 1991, analyzed 1,016 in-flight medical events on U.S. commercial aircraft between 1986 and 1987.⁶² In 123 cases some documentation of the patients' medical history was available. In 76 (62 %) of these 123 cases, the patients had a pre-existing medical condition which was associated with the medical event that occurred on the aircraft. The most common pre-existing conditions were cardiovascular disorders in 31 cases (41%), gastrointestinal disorders in 8 cases (11%), diabetes or other endocrine disorders in 6 cases (8 %), obstetric problems in 5 cases (7%) and allergy conditions in 4 cases (5 %) (Fig. 3). These data point out an important relationship between the medical history and the occurrence of in-flight medical events. They suggest that physicians should pay careful attention to the medical history when evaluating patients for air travel.

The usefulness of pre-flight medical screening of patients with known medical problems was demonstrated in a study published by Gong and associates in 1993.⁵¹ In this study, 1,115 patients were referred by a U.S. airline for medical evaluation prior to flight during calendar year 1991. A total of 42,770,468 passengers flew on this airline during the same period of time. Of the patients referred for pre-flight medical screening, 80 percent had cardiopulmonary conditions that required evaluation for in-flight oxygen therapy. Chronic obstructive pulmonary disease and cardiac disorders were the most frequent problems in this group. The remaining patients consisted of 4.6 percent with major cardiopulmonary disorders not requiring evaluation for oxygen therapy and 15.4 percent with non-cardiopulmonary disorders such as malignancies, neuropsychiatric conditions, orthopedic problems and diabetes mellitus. More than 80 percent of all patients were using prescribed

MEDICAL HISTORY RELATED TO INFLIGHT EVENTS (N=123)

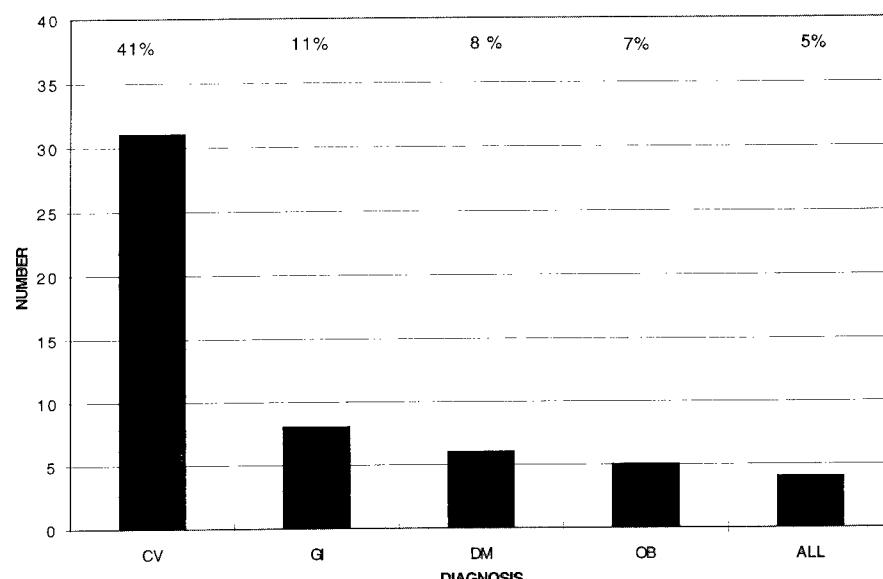


Figure 3 Pre-existing medical conditions related to in-flight medical emergencies: cardiovascular, gastrointestinal, diabetes mellitus, obstetrical, allergy. From Hordinsky and George⁶².

medications. Approximately 90 percent (1,011) of the screened patients were given clearance for air travel, including 92.4 percent of the patients who required supplemental oxygen (all of whom received oxygen during flight). The remaining 104 patients were denied clearance for air travel because of an unstable medical condition or special needs that violated airline policy (e.g. intravenous therapy). Of the 1,011 screened patients who were cleared to fly, none were reported to have experienced a significant in-flight medical problem. The results of this study suggest that pre-flight medical screening can help to determine which patients with pre-existing medical conditions can fly safely on commercial aircraft.

The Aircraft Environment

The environment of commercial aircraft consists of two general components: the external environment and the cabin environment. While these two components are inextricably related, they are quite different in many respects. Therefore, they need to be considered separately. Basic knowledge of the characteristics of both components of the aircraft environment is essential for understanding the effects of commercial air travel on human health.

The External Environment

Most modern commercial aircraft fly at cruising altitudes between 25,000 and 40,000 feet. The time of ascent, cruising altitude achieved and time of descent on any given flight are dependent upon the type of aircraft and the duration of the flight. In general, the flight patterns of commercial aircraft are planned to keep the passengers and crew as safe and comfortable as possible. It must be noted, however, that rapid changes in cruising altitude during emergency maneuvers, sudden turbulence and air-traffic control requirements during ascent and descent can cause abrupt deviations from usual flight patterns. These deviations may place additional physiological stress on both passengers and crew.

As an aircraft ascends to progressively higher altitudes the air density of the atmosphere decreases exponentially. This, in turn, results in an exponential decrease in barometric pressure as altitude increases linearly (Fig. 4). The gaseous composition of the atmosphere, on the other hand, remains remarkably constant up to altitudes of more than 300,000 feet. By dry gas volume, the composition of the atmosphere is 21.0 percent oxygen, 78.1 percent nitrogen, and 0.9 percent argon. The net effect of these phenomena is an exponential decrease in the partial pressure of each atmospheric gas with increasing altitude.^{12,44,115} As shown in Figure 5, the exponential decrease in the partial pressure of inspired atmospheric oxygen (PiO_2) results in a related exponential decrease in the partial pressure of oxygen in arterial blood (PaO_2). At typical cruising altitudes of 25,000 to 40,000 feet the atmospheric PiO_2 should be between 55 and 30 mmHg. The expected PaO_2 at these altitudes should be between 35 and 18 mmHg, respectively.¹⁰² These very low arterial oxygen tensions are incompatible with human life and point out the absolute necessity for pressurization of the aircraft cabin at typical cruising altitudes.

In addition to the decrease in barometric pressure, there is a decrease in atmospheric temperature with increasing altitude. As shown in Figure 6, temperature decreases linearly at a rate of approximately 2°C per 1000 feet of altitude increase up to 40,000 feet. At this point the temperature decrease levels off and remains relatively constant at approximately -57°C up to an altitude of 150,000 feet.^{12,44,115} At

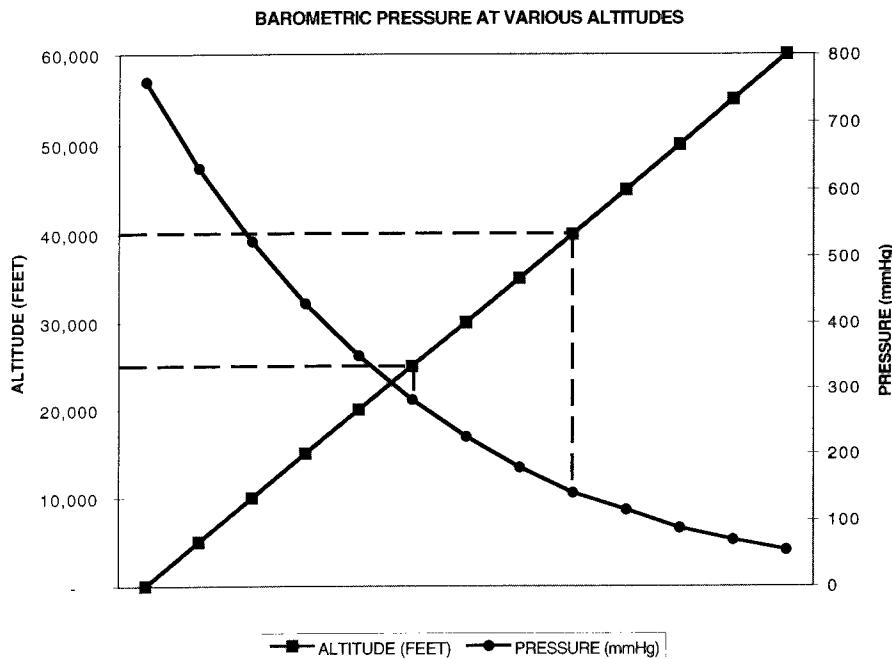


Figure 4 Exponential decrease in barometric pressure with linear increase in altitude. Values at typical aircraft cruising altitudes are indicated by - - - lines.

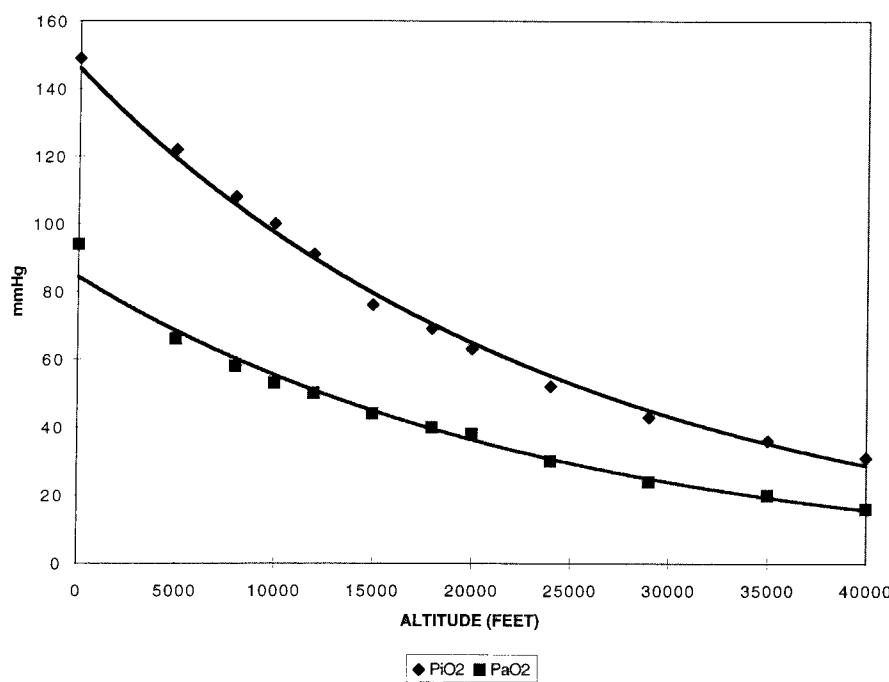


Figure 5 Expected inspired oxygen tension (PiO_2) and arterial oxygen tension (PaO_2) at various altitudes up to 40,000 feet.

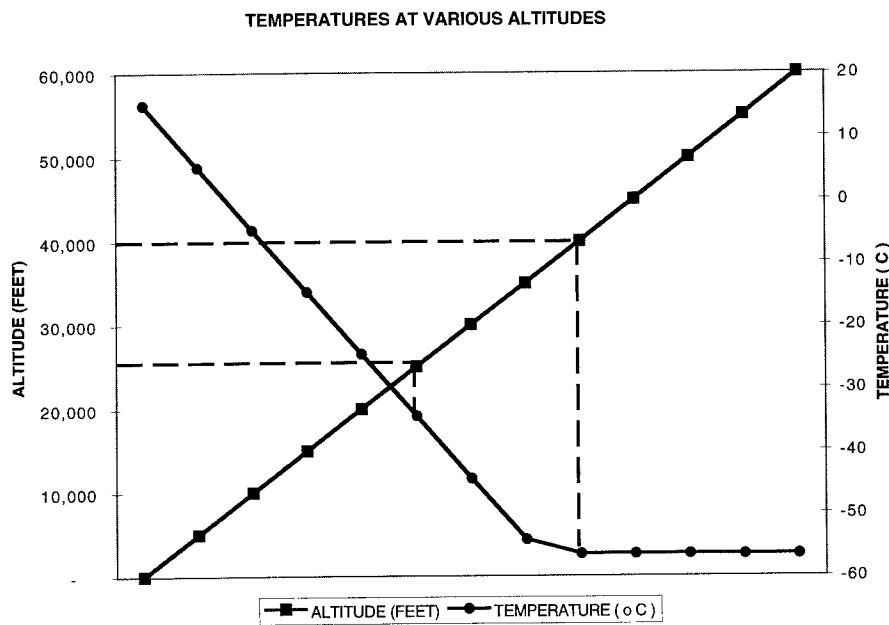


Figure 6 Temperatures at various altitudes up to 60,000 feet.^{12,44,115} Values at typical aircraft cruising altitudes are indicated by --- lines.

typical aircraft cruising altitudes, the atmospheric temperature is between -36° and -57°C . These very low temperatures are also incompatible with human survival. Thus, a highly efficient heat exchange system is required to maintain aircraft cabin temperature at a level which is both safe and comfortable for passengers.

The Cabin Environment

In order to maintain the partial pressures of inspired and arterial oxygen at safe levels, commercial aircraft cabins must be pressurized. Air drawn into an aircraft's jet engines is compressed in superchargers before it is mixed with fuel for combustion. Some of this compressed air is drawn out of the jet engine superchargers and diverted into the aircraft cabin. In commercial aircraft the compressed air is typically piped into the cabin at a relatively high flow rate of 400 liters per passenger per minute. This high rate of airflow helps to remove carbon dioxide, water vapor, noxious gasses and airborne microbes. The pressure in the cabin is regulated by a series of outlet valves. These outlet valves open and close automatically in response to the difference between atmospheric pressure and cabin pressure. In this manner they maintain the cabin pressure within desired limits by controlling the volume of air within the cabin.^{44,59} This type of pressurization system will maintain the cabin pressure at ground level until the aircraft reaches an altitude of approximately 16,000 feet. As the aircraft ascends above 16,000 feet the cabin pressure gradually but steadily decreases. On modern commercial aircraft the cabin pressure decreases at a rate equivalent to a slow climb of 300 feet per minute, even though the actual rate of ascent is normally more rapid.¹⁰³

At typical cruising altitudes between 25,000 and 40,000 feet the cabin pressure remains relatively constant during normal operations until the aircraft begins to

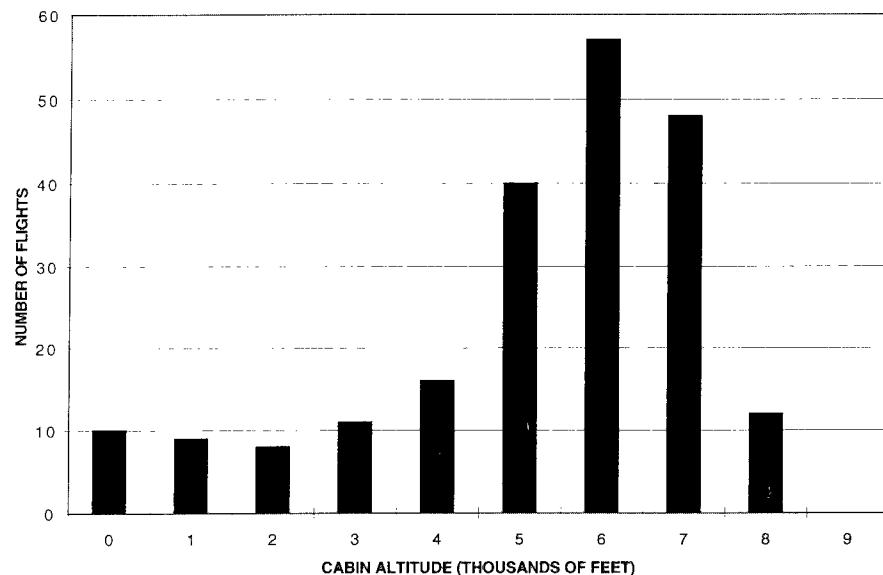


Figure 7 Frequency distribution of aircraft cabin altitudes measured on 240 commercial flights. From Cotrell.²²

descend. The actual cabin pressure depends upon the flight altitude and the type of aircraft. As a result of pressurization, the cabin altitude is significantly less than the actual cruising altitude of the aircraft. In a study conducted on 240 regularly scheduled commercial flights, the measured cabin altitude was usually between 5,000 and 8,000 feet with a mean of 6,214 feet.²² The distribution of cabin altitudes measured in this study is shown in Figure 7. At these cabin altitudes the inspired oxygen tensions (PiO_2) should be between 110 and 120 mmHg. In healthy individuals with a normal hypoxic ventilatory response, this range of PiO_2 should result in arterial oxygen tensions (PaO_2) between 55 and 65 mmHg respectively. At these levels of PaO_2 , the oxygen saturation of hemoglobin in arterial blood (SaO_2) should be between 87 and 95 percent.^{10,50,64}

In one study, the arterial oxygen saturation of passengers was actually measured during flight using a pulse oximeter.¹⁰⁴ Measurements were recorded at altitudes between 900 and 1950 meters and again at altitudes between 2000 and 3300 meters. This study demonstrated that there was a progressive decline in SaO_2 with increasing age at both altitude intervals (Fig. 8). It was also shown that cigarette smokers had approximately the same SaO_2 as non-smokers of similar age at altitudes between 900 and 1950 meters (Fig. 9). At altitudes between 2000 and 3300 meters, however, smokers had significantly lower SaO_2 values compared to non-smokers of similar age (Fig. 10). The regression line for these data shows that cigarette smokers older than 70 years of age are likely to have an SaO_2 of less than 90%. These elderly smokers, it would seem, are especially at risk of developing hypoxia-related complications during commercial air travel.

In addition to pressurization, the temperature of the cabin must be maintained at a safe and comfortable level. When air is compressed in the superchargers of jet

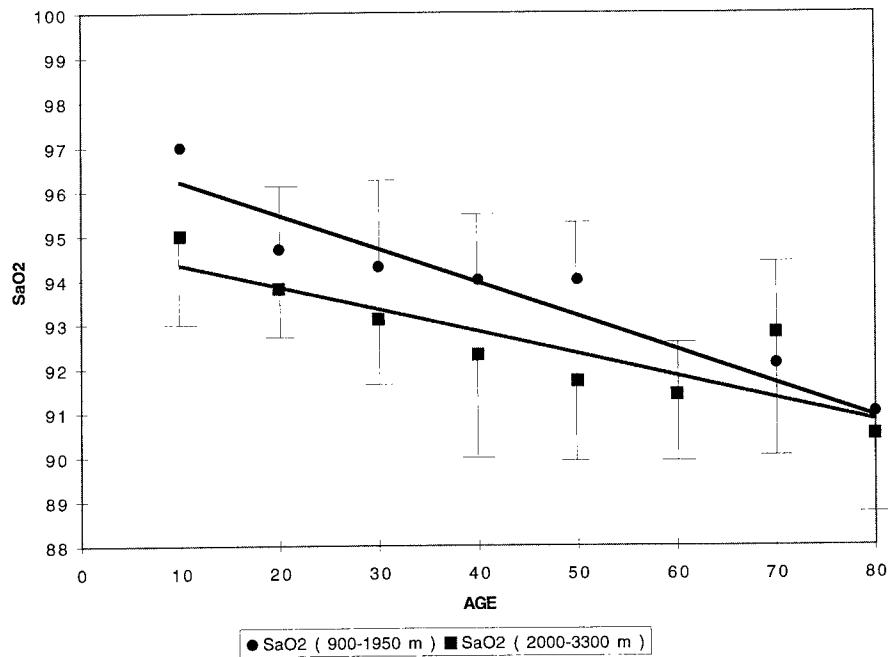


Figure 8 Relationship between arterial oxygen saturation (SaO_2) and age at two different altitude intervals during commercial flights: 900-1950 meters and 2000-3300 meters. From Soskuty.¹⁰⁴

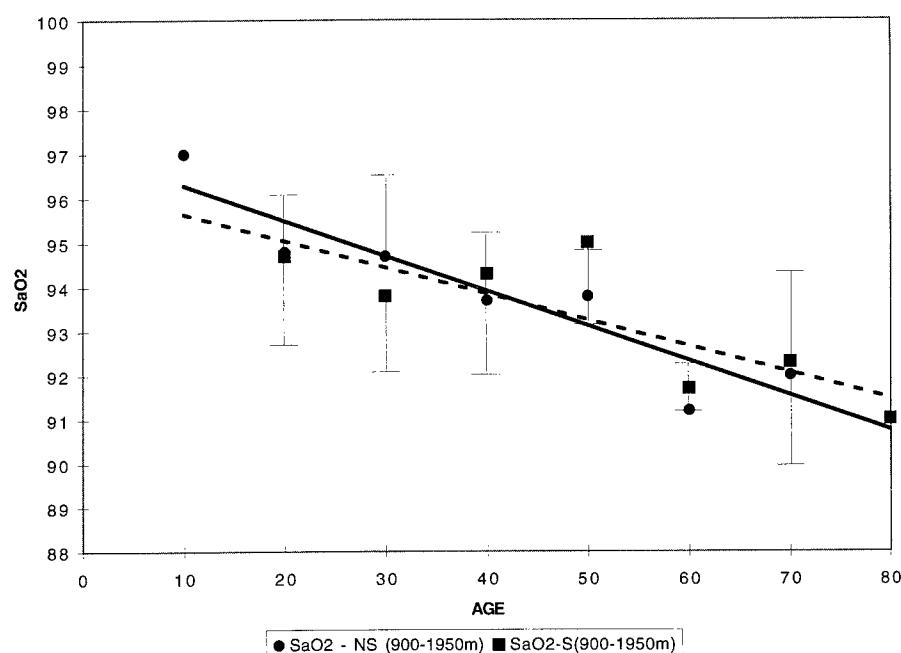


Figure 9 Arterial oxygen saturation of smokers (---) and non-smokers (—) of similar age at altitudes between 900 and 1950 meters during commercial flights. From Soskuty.¹⁰⁴

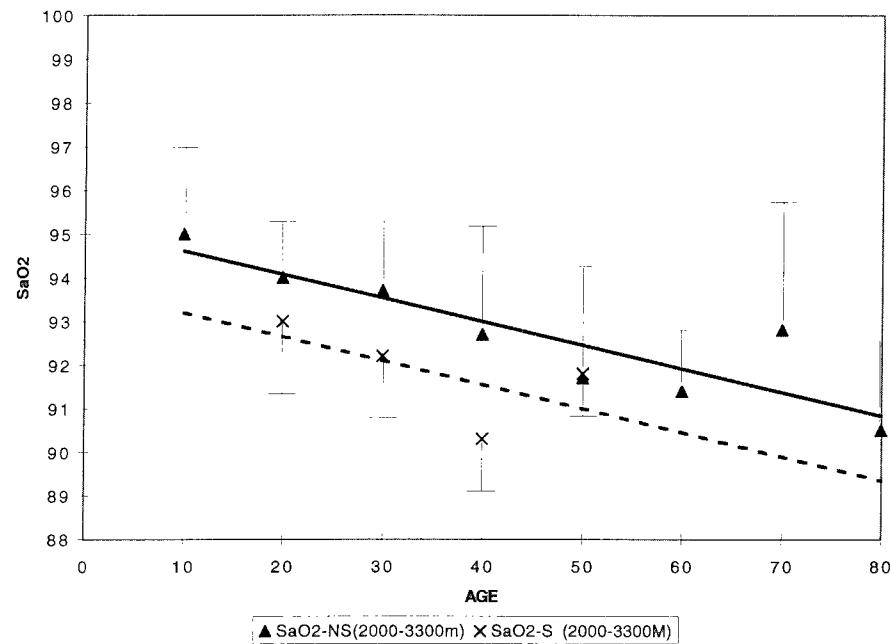


Figure 10 Arterial oxygen saturation of smokers (---) and non-smokers (—) of similar age at altitudes between 2000 and 3300 meters during commercial flights. From Soskutu.¹⁰⁴

engines the temperature of the compressed air increases significantly. The compressed air that is drawn off the superchargers for cabin pressurization has a temperature as high as 315°C.⁵⁹ This hot air is passed through heat exchange units and cooled to room temperature before being piped into the aircraft cabin. The heat exchange units on modern commercial aircraft are very efficient and normally keep the cabin temperature relatively comfortable at 12 to 18°C during all phases of flight.

Cardiovascular Disease

The decreases in PaO_2 and SaO_2 experienced at maximum cruising altitudes have the potential to precipitate significant problems in some patients with cardiovascular disease.^{1,5,10,91,103,109} In general, as PaO_2 decreases below 60 mmHg all individuals experience compensatory increases in both minute ventilation and heart rate. The magnitude of these increases is inversely proportional to the decrease in PaO_2 . An increase in minute ventilation results in an increase in work of breathing. An increase in heart rate causes an increase in myocardial oxygen demand. Healthy individuals and, indeed, most patients with cardiovascular disease, have sufficient cardiopulmonary reserve to meet these physiological stresses during air travel. However, patients with low baseline PaO_2 , respiratory muscle weakness, or conditions in which myocardial oxygen demand exceeds myocardial oxygen supply may experience decompensation of their underlying cardiovascular disorders during flight. In an effort to prevent in-flight cardiovascular emergencies, guidelines for air travel have been developed for patients with angina pectoris, myocardial infarction, coronary artery bypass surgery and congestive heart failure.^{1,50,103}

Angina Pectoris

Unstable angina is an *absolute contraindication* for commercial air travel.¹

Patients with stable angina pectoris (Class I and II) can fly safely on commercial aircraft as long as they carry their medications and take them as prescribed. It is recommended that patients with more severe, but stable, angina (Class III and IV) use supplemental oxygen during commercial air travel in addition to taking their usual medications as prescribed.¹ These classifications of angina are defined as follows:¹⁸

Class III "Marked limitation of ordinary physical activity.

Walking one to two blocks on the level and climbing one flight of stairs in normal conditions and at normal pace."

Class IV "Inability to carry on any physical activity without discomfort - anginal syndrome *may* be present at rest."

Supplemental oxygen should be administered at low flow rates (2 liters per minute) during flight.

Myocardial Infarction

Patients with recent, *uncomplicated myocardial infarctions* should not fly for at least three weeks from the onset and until they can perform their usual daily activities without difficulty.^{1,4} At three weeks after myocardial infarction all such patients should have a symptom-linked exercise test.^{4,42} They can be cleared to fly if there are no symptoms and no evidence of myocardial ischemia at maximal exercise. Cleared patients must continue their usual medications during flight, which should include a beta-adrenergic blocker for Q-wave infarction and a calcium channel blocker for non-Q-wave infarction.⁹⁵ Patients who are symptomatic or demonstrate signs of myocardial ischemia during exercise should undergo further medical evaluation and should not fly until their condition is entirely stable.

Individuals who have suffered a recent *complicated myocardial infarction* should not fly for at least six weeks.¹ Beyond that period of time, they should not fly until their medical condition has stabilized on appropriate medication for at least three weeks and they are free of symptoms, dysrhythmias, hypotension or signs of ischemia on exercise testing.

Patients with old, *asymptomatic* myocardial infarctions can generally fly safely.¹ On the other hand, patients with old infarctions who have angina, evidence of ventricular dysfunction or a history of cardiac dysrhythmia should undergo a thorough medical examination prior to being cleared for air travel.

Coronary Artery Bypass Surgery

Patients who have undergone successful coronary artery bypass surgery and have fully recovered without complications can fly without increased risk. It is recommended that these patients wait at least two weeks before flying to allow for complete resorption of any air in the chest cavity as a result of surgery.^{1,5} It is also recommended that these patients undergo a thorough medical examination, to include exercise testing, prior to being cleared for air travel. Any evidence of residual ischemia, ventricular dysfunction, or serious dysrhythmia are relative contraindications to air travel in this group of patients.

Percutaneous Transluminal Coronary Angioplasty

Patients who have undergone uncomplicated percutaneous transluminal coronary angioplasty (PCTA) can safely travel by air after one to two weeks if they are asymptomatic, medically stable and can perform their usual activities.¹ If there were complications associated with the procedure or the patient was unstable pre-operatively, a medical examination should be performed to ascertain hemodynamic stability prior to commercial air travel.

Congestive Heart Failure

Decompensated congestive heart failure is an absolute contraindication for commercial air travel.^{1,5,103} Patients with New York Heart Association Class III or Class IV congestive heart failure should be given in-flight supplemental oxygen during air travel.¹ These classifications are defined as follows:²⁵

Class III "Patients with cardiac disease that results in marked limitation of physical activity and causes fatigue, palpitations, dyspnea or anginal pain."

Class IV "Patients with cardiac disease that results in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present at rest. If any physical activity is undertaken, discomfort is increased."

Patients with less severe congestive heart failure may travel by air as long as they are medically stable on their medication and their baseline PaO₂ is 70 mmHg or greater. If the baseline PaO₂ is less than 70 mmHg, supplemental oxygen should be prescribed.

Valvular Heart Disease

Symptomatic valvular heart disease, regardless of etiology, is a relative contraindication for commercial air travel.¹ These patients should undergo a thorough pre-flight medical examination. Clearance to fly should be given only after the cause of symptoms has been ascertained and corrected, the patient is hemodynamically stable, and any dangerous dysrhythmias have been adequately treated. If baseline PaO₂ is less than 70 mmHg, supplemental oxygen should be administered during flight.

Patients with asymptomatic valvular heart disease can generally fly safely if they are hemodynamically stable and continue their usual medications. However, individuals with mitral stenosis and pulmonary hypertension are at increased risk of cardiovascular decompensation during air travel, even if asymptomatic. Patients with mild to moderate pulmonary hypertension should receive in-flight supplemental oxygen at flow rates of 2 to 4 liters per minute.⁵⁰ Patients with severe pulmonary hypertension should not travel by air.

Congenital Heart Disease

The Aerospace Medical Society currently recommends that passengers with cyanotic congenital heart disease (CCHD) receive supplemental oxygen during commercial air travel.¹ However, an interesting study by Harnick and associates

questions the need for in-flight supplemental oxygen in adults with CCHD.⁵⁸ In this study, 12 adult patients with various types of CCHD and 27 control subjects were evaluated during simulated commercial flights in a hypobaric chamber. In addition, 10 patients and 6 control subjects were evaluated during two actual flights of approximately 2.5 hours each. There was no statistically significant decrease in capillary blood PO₂ in the patient group during actual flights, compared to a mean decrease of 15 mmHg in the control group during the same flights. Although mean pre-flight SaO₂ of the patient group was lower than that of the control group, the percentage decrease in SaO₂ during simulated flights was essentially the same in both groups. The authors speculate that a high level of erythrocyte 2,3-DPG and a blunted hypoxic ventilatory response may cause a rightward shift of the oxyhemoglobin dissociation curve in patients with CCHD. They suggest that adult patients with CCHD may not require supplemental oxygen during commercial air travel. The authors caution, however, that this should not be an "automatic" recommendation for all patients with CCHD at this point in time. More studies are required before this issue can be resolved with certainty.

In general, patients with acyanotic congenital heart disease can travel safely by air as long as they are hemodynamically stable and continue their usual medications. However, Eisenmenger complex, (characterized by congenital heart disease with pulmonary hypertension and right-to-left shunt)⁹⁸ has been reported to be an absolute contraindication for commercial air travel.¹

Deep Venous Thrombosis

Commercial air travel is an established risk factor for the development of deep venous thrombosis (DVT).^{11,26,68} While the exact incidence of DVT related to air travel is unknown, there is one report of 8 to 10 percent of all cases admitted to the Auckland area hospitals occurring after travel of long duration.¹⁰⁷

Travel on commercial aircraft produces a variety of conditions which increase the risk of developing deep venous thrombosis in the lower extremities. Sitting in a cramped position for long periods of time will increase venous pressure and may cause stasis of venous blood flow in the feet and legs. Damage to the venous endothelium may be caused by direct pressure on leg veins by the edge of the passenger seat.¹ It is also known that the decreased barometric pressure of the aircraft cabin causes fluid shifts from the intravascular space into the interstitial space of the lower extremities.⁶⁸ The resulting hemoconcentration can cause an increase in blood coagulability. The combined effects of these phenomena provide an ideal pathophysiological substrate for the development of deep venous thrombosis during commercial flight.

The Aerospace Medical Association has published guidelines for the prevention of DVT during commercial air travel.¹ These are summarized below:

- Do not place baggage under the seat in front to minimize cramping of the legs.
- Exercise the legs at regular intervals. This should include periodic stretching and flexing of the feet and short walks through the cabin once each hour.
- Do not sleep in a cramped position.
- Do not use hypnotic drugs during flight. These may cause muscle relaxation which promotes venostasis. Deep sleep may also lead to unrecognized, severe compression of leg veins.
- Consume fluids both before and during flight.

- Avoid alcoholic beverages. The diuretic and vasodilating effects of alcohol may contribute to hemoconcentration and venostasis.

Certain individuals may be at high risk for developing DVT during air travel. These include patients with a history of previous DVT, malignancy, immobility of the lower extremities, or recent surgery. It is recommended that such individuals wear compression stockings and receive subcutaneous heparin (5,000 units) before and after flight. Patients with a history of multiple DVT or pulmonary embolism should be fully anticoagulated with warfarin prior to air travel.¹ Pregnant women in this category should be treated with subcutaneous heparin rather than warfarin.

Pulmonary Disorders

Some patients with pulmonary disorders may experience symptomatic decompensation of their illnesses as a result of a decrease in PaO₂ during air travel.^{8,38,39,50,74,91,100,103,109} The most important factors which must be considered in the evaluation of such patients are: (1) the baseline PaO₂ at ground altitude; (2) functional severity of the disorder; (3) degree of reversibility of the disorder; and (4) the pulmonary and respiratory muscle reserve to sustain an increase in minute ventilation as a compensatory response to hypoxia. The clinical and laboratory evaluation of these factors can help the physician determine which patients can fly safely, which patients require in-flight supplemental oxygen and which patients should not fly at all.

Chronic Obstructive Pulmonary Disease

Individuals with chronic obstructive pulmonary disease (COPD) are especially susceptible to respiratory decompensation during commercial air travel.^{6,8,38,39,49,103} In general, the risk of decompensation increases with increasing severity of disease. Thus, all patients with COPD should have a thorough medical examination prior to flight. This examination should include a detailed medical history, physical examination, pulmonary function testing and arterial blood gasses.

The following parameters can be used to identify COPD patients who are at high risk for respiratory decompensation during commercial air travel:^{6,100}

- Dyspnea on mild exertion (e.g. walking 50 yards)
- Predicted in-flight PaO₂ of less than 50 mmHg
- Maximum voluntary ventilation (MVV) less than 40 L/min
- CO₂ retention

Patients who meet *any one* of the above criteria should be treated with supplemental oxygen during flight. Patients not already treated with oxygen at ground altitude should receive in-flight oxygen at a flow rate of 2 liters per minute. Those who are already treated with supplemental oxygen should have their baseline flow rate increased by 33 percent.⁵¹ The goal of oxygen therapy should be to maintain the in-flight PaO₂ greater than 50 mmHg.^{6,13,50}

Prediction of the in-flight PaO₂ can be a very useful tool in the evaluation of COPD patients for air travel. As a rough estimate, COPD patients with a baseline PaO₂ less than 67 mmHg can be expected to have an in-flight PaO₂ less than 50 mmHg at typical cabin altitudes.¹⁰⁰ In another report, a baseline PaO₂ of 68 mmHg had a good correlation with a PaO₂ less than 55 mmHg at an altitude of 5,000 feet and a baseline PaO₂ of 72 mmHg had a good correlation with a PaO₂ less than 50

mmHg at an altitude of 8,000 feet.⁴⁷ More accurate predictions can be obtained by several formulas which have been reported in the literature.^{13,38,81,100} One such formula, developed through multivariate regression analysis, is as follows:⁸¹

$$P_aO_2[ALT] = 0.19(FEV_1 * P_aO_2[GND]) - 11.51[\ln(MA-GA)] + 123.17$$

where $P_aO_2[ALT]$ is the predicted arterial oxygen tension at moderate altitude; FEV_1 is the forced expiratory volume of air in one second during spirometric testing at ground altitude; $P_aO_2[GND]$ is the baseline arterial oxygen tension measured at ground altitude; MA is the maximum altitude in meters (e.g. cabin altitude) to which the individual will ascend; and GA is the ground altitude at which baseline measurements were made. This formula has a very high predictive value ($r^2 = 0.99$) and a high level of statistical significance ($p = 0.01$) at moderate altitudes. Calculations can be performed quite easily with a scientific calculator or a computer spreadsheet.

In patients with complicated, hypoxic COPD a high altitude simulation test may be considered as part of the pre-flight evaluation.⁴⁷ In this procedure the patient breathes hypoxic gas mixtures which simulate the PiO_2 at typical cabin altitudes. The hypoxic gas mixture is administered alone and with the addition of supplemental oxygen. The PaO_2 and $PaCO_2$ at simulated cabin altitudes, the changes in PaO_2 and $PaCO_2$ with supplemental oxygen, the breath-by-breath ventilatory response, pulse and blood pressure responses and electrocardiographic changes can then be measured.

Asthma

Asthma is the most common respiratory disorder among commercial air travelers.¹ Patients with severe or poorly controlled asthma should not travel by air. Those who have had a recent hospitalization should refrain from air travel until their condition is entirely stable and well-controlled with medication.

In general, asthmatics with a stable condition that is well-controlled with medication can fly safely. Such individuals must continue all medication as prescribed and carry all medication with them during travel. It is also recommended that they carry a course of oral corticosteroids for emergency use during flight.¹

Pulmonary Hypertension

Patients with pulmonary hypertension, either primary or secondary to other disorders, are at high risk for complications during commercial air travel. Even a mild increase in hypoxia during flight may precipitate significant increases in pulmonary vascular resistance and pulmonary arterial pressure. These changes may, in turn, cause a life-threatening decrease in cardiac output.^{3,50,96}

Severe or hypoxic pulmonary hypertension is an absolute contraindication for air travel. Patients with mild to moderate pulmonary hypertension should receive in-flight supplemental oxygen at flow rates of 2 to 4 liters per minute. It is essential for these patients to continue all prescribed medications, to include anticoagulants. They should also be advised to keep exertion to an absolute minimum during flight.

Pneumothorax

The presence of an active pneumothorax is an absolute contraindication for air travel.^{1,4} Any air in the chest cavity may expand during flight and progress to a tension pneumothorax with life-threatening cardiopulmonary compromise.^{5,49} It is currently recommended that patients wait at least 2 to 3 weeks after successful

drainage of a pneumothorax or chest surgery before traveling by air.¹ In most cases this period of time should be adequate for healing of the underlying disorder and the reabsorption of any residual air. It would, however, be prudent to obtain a follow-up chest x-ray prior to flight to exclude the presence of residual air or recurrence of the pneumothorax.

Neurological Disorders

As PaO_2 decreases cerebral blood flow increases. Conversely, as PaCO_2 decreases cerebral blood flow decreases. These two metabolic regulators of cerebral blood flow counteract each other during hypocapnic hypoxia.^{92,113} In general, PaCO_2 is a more potent metabolic regulator of cerebral blood flow than PaO_2 .^{69,106} It has also been shown that the most significant effects of PaO_2 on cerebral blood flow do not occur until the PaO_2 falls below 50 mmHg.⁶⁷ At typical cabin altitudes individuals with normal gas exchange would be expected to have a PaO_2 in the range of 55 to 65 mmHg and a PaCO_2 in the range of 36 to 39 mmHg.^{10,50,64} At these levels the PaCO_2 would appear to be the predominant metabolic regulator and cause a mild net decrease in cerebral blood flow. This could have an adverse effect on patients with a recent stroke or a history of seizure disorders.

Stroke

Patients who have had a recent stroke should wait at least 2 weeks from the time that their condition stabilizes before traveling by air.¹ Such patients should have a thorough medical examination and be hemodynamically and neurologically stable prior to flight. They must continue their medication as prescribed and address any special needs, such as wheelchair assistance, with the airline prior to departure.

Seizure Disorders

The combined effects of hypoxia, mildly decreases cerebral blood flow and altered circadian rhythm may lower the threshold for seizure activity during commercial air travel.^{55,66,77} Therefore, patients with frequent or uncontrollable seizures should not travel by air. Those with a history of infrequent seizures or loss of consciousness are advised to travel with a companion.¹ It is essential for patients being treated for a seizure disorder to continue their medication exactly as prescribed. They should also avoid alcoholic beverages both before and during flight, since alcohol can lower the threshold for seizure activity.³³

Pregnancy

Numerous studies have demonstrated that air travel causes no increased risk to the mother or the fetus during normal pregnancy.^{9,15,16,29} At typical cabin altitudes maternal hemoglobin is normally more than 90 percent saturated.^{1,90} Fetal Hemoglobin F has a lower affinity for 2,3-DPG than adult Hemoglobin A, resulting in a shift of the oxyhemoglobin dissociation curve to the left.⁹⁹ The increased oxygen saturation of Hemoglobin F leaves fewer H^+ binding sites on deoxygenated hemoglobin which lowers the pH of fetal blood and shifts the oxyhemoglobin dissociation curve to the right.⁹⁰ The net effect of these opposing phenomena is no change in fetal PaO_2 at typical aircraft cabin altitudes.¹

Clinical reports have shown that there is no increase in the risk of spontaneous abortion and no significant alteration in fetal physiology during air travel.^{63,76}

Furthermore, several studies have demonstrated that chronic exposure to hypobaric hypoxia at typical cabin altitudes causes no adverse effects during normal pregnancy.^{1,17,30}

Special precautions do need to be taken for air travel during complicated pregnancy. First of all, it is recommended that any significant pregnancy-related anemia be corrected prior to flight. Furthermore, any condition which causes a decrease in fetal-placental oxygen reserve is a relative contraindication for commercial air travel. Such conditions include pre-eclampsia, intrauterine growth retardation, post-maturity syndrome and placental infarction. Pregnant women with such disorders should undergo a thorough pre-flight medical examination. In-flight supplemental oxygen should be prescribed if they are determined to be stable enough for air travel.¹

All women who fly during pregnancy should be advised to wear their seat belts continuously while seated. The seat belts should be worn over the lower pelvis or upper thighs. These measures will help to prevent abdominal trauma during unexpected turbulence.¹ Air travel after 36 weeks gestation needs to be considered on an individual basis. More than 90 percent of all spontaneous deliveries occur after this point in pregnancy. It is also extremely difficult to predict exactly when the onset of labor will occur.^{1,5,16,29,103} In general, it is recommended that women with multiple pregnancies, cervical incompetence, uterine bleeding, or increased uterine contractions avoid air travel after 36 weeks gestation.¹

Hematological Disorders

Anemia

A hemoglobin concentration of less than 8.5 gm/dL, regardless of the etiology, is a relative contraindication for commercial air travel. At this level of hemoglobin patients may become lightheaded or lose consciousness with mild exertion during flight.^{1,5,103} There may also be an increase in heart rate and stroke volume which could precipitate high output heart failure or an episode of angina in patients with otherwise stable coronary artery disease.^{34,70} Thus, all patients with a hemoglobin concentration below this critical level should have a thorough medical examination before being cleared to fly. Even if they are determined to be altogether stable enough to tolerate the stresses of air travel, it would be prudent to consider the administration of in-flight supplemental oxygen.^{1,103} It is recommended that patients with severe anemia caused by active bleeding from any site not be cleared for commercial air travel.

Sickle Cell Disease

Erythrocytes in patients with sickle cell anemia (i.e. homozygous SS genotype) typically start to sickle at a PaO₂ of approximately 40 mmHg.¹⁴ This is significantly less than the PaO₂ expected at typical cabin altitudes. However, occasional cases of sickle cell crisis have been reported at cabin altitudes normally experienced during commercial air travel.^{14,40,53,54} Although such crises are uncommon, it is recommended that individuals with sickle cell anemia receive supplemental oxygen during flight.¹

Erythrocytes in patients with sickle cell trait (i.e. heterozygous SA genotype) do not start to sickle until a PaO₂ of 15 mmHg is reached.¹⁴ Multiple studies have demonstrated that these patients should tolerate the mild hypoxia of commercial air

travel without difficulty.^{36,37,53,54,114} Therefore, no special precautions for this group of patients need to be taken during flight.

Heterozygous sickle cell-hemoglobin C disease and sickle cell-beta thalassemia seem to be somewhat more severe than sickle cell trait, but less severe than homozygous sickle cell anemia.¹⁴ Indeed, a few individuals with sickle cell-hemoglobin C disease have been reported to experience painful crises at altitudes typically experienced during commercial air travel.^{14,53} Recommendation of in-flight supplemental oxygen for these patients should be guided by the past medical history. Those who have experienced any pain or discomfort during air travel or ascent to high terrestrial altitudes should receive supplemental oxygen. Those without a history of altitude-related symptoms can probably travel without supplemental oxygen.

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CHAPTER 28

SLEEP APNEA LEADS TO CHRONIC HYPERTENSION VIA INTERMITTENT HYPOXIA

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Sleep predisposes to disordered breathing (SDB) across a broad continuum ranging from periodic episodes of transient hypoventilation to full-blown obstructive sleep apnea syndrome (OSA). Epidemiologic studies of a large working population, aged 30 to 60 years, showed that apneas and hypopneas occurred at a rate ≥ 10 to 15/hr in 4% of females and 9% of males.²⁶ The great majority of the events in this undiagnosed population are hypopneas (or transient underventilations) with relatively few apneas.

Causes of Sleep Disordered Breathing

A major cause of SDB is a sleep-induced reduction in tonic neural activity to "stiffening" muscles of the upper airway, especially in REM sleep. Thus, in most healthy adults, compliance of the upper airway increases in sleep and the negative intrathoracic (downstream) pressure developed during inspiration causes airway narrowing.²² The resultant increase in resistance of the upper airway contributes - along with the loss of wakefulness and its associated neural input to medullary respiratory neurons - to moderate levels of alveolar hypoventilation (+2 to +8 mmHg PaCO₂) experienced by all of us during sleep.¹² Because sleep state changes periodically throughout the night, as does body posture, neck flexion, lung volumes and caudal traction on the trachea, so does resistance of the upper airway. These transient increases in upper airway resistance do not provoke an immediate compensatory increase in the neural drive to breathe during sleep; thus, they are a common cause of reductions in flow rate and intermittent hypopneas.¹⁴ In persons with already compromised airway structure, commonly because of increased body mass or craniofacial abnormalities, sleep and changing sleep states result in marked, intermittent airway narrowing and often airway closure.

There are also several other forms of sleep disordered breathing which are not caused primarily by compromised upper airway patency. These include: a) **central apnea** - which is caused by transient cessations of central respiratory neural drive. These apneas commonly follow a transient ventilatory "overshoot"- induced by changes in sleep state or airway resistance.^{4,25} They are attributable primarily to hypocapnia, because during sleep, just a few mmHg sustained reduction of PaCO₂

below eupnea is sufficient to markedly inhibit and sometimes completely stop the drive to breathe;⁶ b) **Cheyne-Stokes respiration**, consisting of alternating waxing and waning of tidal volume especially during non-REM sleep and particularly in patients in chronic heart failure; and c) the “cluster” breathing experienced by most sojourners during sleep at high altitudes, where central apneas alternate with groups of 2-5 breaths at huge tidal volumes with amazing regularity.² All of these types of episodic ventilatory insufficiencies cause intermittent asphyxia, chemoreceptor stimulation, increased respiratory efforts, and transient arousals.

Consequences of Sleep Disordered Breathing

What are the acute and chronic consequences of these transient, repetitive events? How severe must SDB be in order to have pathobiological significance in terms of long-term sequelae? These questions are not yet resolved; in other words, the disease “threshold” for SDB has really not yet been defined in this multi-faceted syndrome. Recently however, a few clues have emerged.

First, it is well established that **multiple arousals** and **sleep state discontinuity** do result from sleep disordered breathing or even from intermittent increases in upper airway resistance alone without apnea.¹⁰ These cortical arousals probably result from sensory inputs to the cerebral cortex caused by chest wall muscular efforts. In turn, the sleep deprivation caused by these arousals - especially REM sleep deprivation - leads to daytime hypersomnolence and significant interference with the individual’s ability to function at work, socially, and behind the wheel. Treatment of sleep apnea - most effectively via mechanical splinting of the hypotonic upper airway with nasal continuous positive airway pressure (CPAP) - results in almost immediate alleviation of daytime hypersomnolence and marked improvement in well being.

Secondly, OSA has also been implicated in the **exacerbation of some chronic cardiopulmonary diseases**. For example, in patients with chronic obstructive pulmonary disease, nightly OSA with intermittent hypoxemia and hypercapnia might - acting via chemoreceptor “resetting” - be an important determinant of daytime chronic respiratory failure, i.e., so-called “**overlap syndrome**”.⁷ Congestive heart failure (CHF) is also worsened by the negative intrathoracic pressure and therefore increased ventricular transmural pressure and increased ventricular afterload generated by inspiratory efforts against an obstructed airway. Accordingly, treatment of OSA (via CPAP) has recently been shown to greatly improve ventricular ejection fraction and clinical status in CHF patients.²⁰

More recently, much focus has been placed on the potential **cardiovascular sequelae of sleep disordered breathing**. The cardiovascular responses to each apneic or hypopneic event are analogous to a defense homeostatic response. Cardiac output, stroke volume and blood pressure decline slightly during the apneic event - especially during an obstructive event with progressive negative pressure being developed against the upper airway obstruction - and then systemic pressure increases abruptly and transiently upon termination of each event, often accompanied by overshoots in ventilation and heart rate, an arousal and a reduced stroke volume.⁹ The key mechanism underlying these transient pressor responses is sympathetically mediated vasoconstriction. Note in Fig. 1 the increase in muscle sympathetic nerve activity, which begins to rise during the apnea and peaks immediately following each event. It is also known that ganglionic blockade prevents the pressor response to

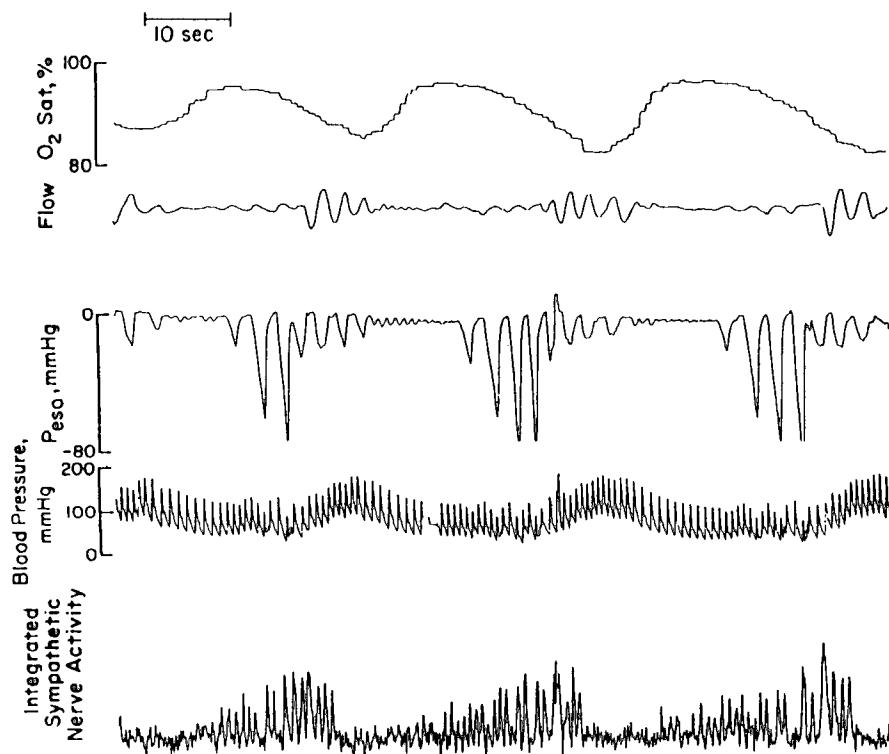


Figure 1 Patient with severe sleep disordered breathing. Mixed apneas (central followed by obstructed) are shown and each apnea is followed by a brisk hyperpnea, an augmentation of muscle sympathetic nerve activity (measured with an intraneurial electrode in the peroneal nerve) and a rise in blood pressure. Respiratory drive is then reduced and a central and then obstructive apnea follow, as the cycle repeats itself. Central apneas are defined by no airflow combined with no respiratory "effort", i.e., esophageal pressure is unchanging; whereas obstructed apneas also have no flow, but decreased esophageal pressure or increased inspiratory efforts. A transient cortical EEG arousal (not shown) occurred at the termination of each obstructed apnea.

apnea.²¹ In turn, the increase in sympathetic outflow is mediated by asphyxic chemoreceptor stimulation - primarily carotid chemoreceptors (via hypoxia and increased CO₂) and central chemoreceptors (increased CO₂); and also possibly by higher hypothalamic central influences on vasomotor medullary cells triggered by arousal. In addition to the transient increase in blood pressure with each SDB event, a more sustained consequence is the absence of the normal lowering effect of sleep on blood pressure. There is also recent evidence that repetitive intermittent hypoxia for 20 minutes in humans causes a sustained increase in muscle sympathetic nerve activity that persists for an hour or so after the hypoxia is withdrawn.¹⁷ This short-term memory or carryover effect of SDB on sympathetic activity may account for the persistence of high systemic blood pressure throughout the night.

Evidence linking Sleep Disordered Breathing with Chronic Hypertension

Mounting evidence of several types points strongly to sleep apnea as a significant cause of daytime hypertension. In other words, recurring episodes of

hypertension over about a third of the twenty-four hour day may be translated into a persistent abnormality.

- Systemic hypertension and cardiovascular morbidity have been linked statistically to obstructive sleep apnea in **population studies**.^{13,27} These findings are controversial because it is difficult to prove these associations in cross-sectional studies which are confounded with the presence of common risk factors between OSA and hypertension such as age, gender, obesity and alcohol consumption. Nevertheless, in a very large undiagnosed cohort, sleep disordered breathing was shown to contribute independently of these other risk factors to significant daytime (and nighttime) elevations in systemic blood pressure and to the prevalence of systemic hypertension.^{13,27}

- **Treatment of OSA** in many (but not all) patients using nasal CPAP was shown to reduce daytime systemic blood pressure.²⁴ A confounding factor in these clinical data is that the reversibility of the hypertension via OSA treatment may be significantly dependent upon whether vascular remodeling has taken place.

- Several weeks of **episodic hypoxia in rats** led to chronic systemic and pulmonary hypertension after the hypoxemia was removed.^{8,19} Furthermore, in the healthy human, systemic blood pressure rises during sojourn in the hypoxia of high altitude and remains elevated for several days on return to sea level.¹

- Additional recent experimental evidence has been produced in a unique **canine model of OSA** using repeated intermittent tracheal occlusions, i.e. obstructive apneas, at a rate of 40 to 60/h for up to 14-16 h/day.³ These apneas increased average night time blood pressure about 10% above control and caused significant increases in daytime blood pressure which were apparent as early as two weeks, peaked at five weeks and recovered to normal within three weeks of return to uninterrupted sleep. This experimental animal model represents the very extreme in severity of human sleep disordered breathing.

So, taken together these cross-sectional data and treatment outcomes in human populations and experimental data in animal models which have simulated all or part of the characteristics of sleep apnea, show conclusively that intermittent hypoxia and/or apnea can clearly lead to permanent systemic - and in some cases pulmonary - hypertension.

Sleep Disordered Breathing → Daytime Hypertension: Mechanisms?

The primary “trigger” mechanisms responsible for the conversion of the acute repetitive pressor responses to nocturnal apneas into persistent daytime hypertension have not been elucidated. Three potential triggers include arousal, hypoxic chemoreceptor stimulation and negative intrathoracic pressure.

Transient arousal, per se, is a promising mechanism because arousals certainly do contribute significantly to the acute pressor response following many apneas. Furthermore, acoustically induced non-apneic arousals - by themselves - have also been shown to cause small transient increases in sympathetic outflow and blood pressure in sleeping humans.¹⁶ However, the recent study by Brooks, et al.³ (see above) showed that many weeks of acoustically induced arousals and sleep discontinuity, per se, sufficient to increase night time blood pressure to the same extent as repetitive apneas, did not cause the sustained increase in daytime blood pressure.

The effects of repeated long-term **negative intrathoracic pressure** swings alone have not been studied. However, acute production of negative intrathoracic pressure in humans has been shown to reduce blood pressure and sympathetic nerve activi-

ty.¹⁸ Furthermore, central apneas (i.e., without inspiratory effort) elicit increases in blood pressure and sympathetic outflow similar to those with obstructive apnea.^{8,18}

The most likely primary trigger mechanism to elicit long-term increases in daytime blood pressure is **intermittent hypoxia acting via increased carotid chemoreceptor stimulation of the sympathetic nervous system**. Thus, acute increases in blood pressure and sympathetic outflow in response to obstructive apnea are prevented via hypoxia.¹⁸ Furthermore, following carotid chemoreceptor denervation or chemical sympathectomy, rats did not become hypertensive upon exposure to long-term, intermittent hypoxia.⁸ In addition, human OSA patients with hypertension have elevated levels of muscle sympathetic nerve activity and circulating catecholamines, which are often reduced with treatment and these patients display augmented responses of blood pressure and ventilation to acute hypoxia.¹¹

Long-term effects on microvascular tone and blood pressure may be due to several types of **chemical modulators** of vasomotor activity.^{8,11} These might include up-regulation of the renal or endothelial renin-angiotensin system or down-regulation of nitric oxide-induced vasodilator tone. These potential mechanisms remain to be investigated; however, the negative findings obtained by Brooks, et al.³ with acoustic arousals (see above) cast doubt on the popular premise that repetitive contraction/relaxation of vascular smooth muscle, *per se*, is the critical factor in the release of these mediators or in their long-term effects which persist beyond the period of intermittent, nocturnal events.

Hypertension → Sleep Disordered Breathing?

The evidence cited above demonstrates that OSA can cause hypertension, but the converse - that cardiovascular disease promotes SDB - may also be true. Transient elevations in systemic blood pressure have been shown to depress ventilation, to reduce electromyographic activity of the muscles of the upper airway, and to increase pharyngeal collapsibility; whereas sustained decreases in blood pressure stimulate ventilation.^{8,23} Furthermore, some types of OSA are ameliorated after treatment of high blood pressure.¹⁵ Thus, these findings raise the intriguing possibility that the acute and chronic hypertension caused by OSA can in turn exacerbate OSA; the two diseases may be linked via positive feedback. Similarly, Cheyne-Stokes respiration and central apneas in CHF are likely precipitated by ventricular dysfunction, in part due to increased circulation time, but perhaps even to a greater extent by the long-term hyperventilation (i.e., hypocapnia) in these patients, caused by their increased pulmonary capillary and interstitial fluid pressures.²⁰

Clinical Relevance

Several important clinical considerations are forthcoming from the mounting evidence supporting a causal relationship between OSA and hypertension. First, it seems that an as yet unquantified but **significant portion of “essential hypertension” is probably attributable to SDB**. This represents a significant public health burden that deserves intense investigation. Given the cumulative severity of episodic hypoxia (and probably sympathoexcitation) induced during the periodic breathing in **sleep at high altitudes**, it is also not unreasonable to suspect that SDB represents an even greater risk factor for hypertension (systemic and pulmonary) in permanent residents at high altitude. Second, it is important to realize that the usual daytime screening which might indicate only “borderline” hypertension must often **underes-**

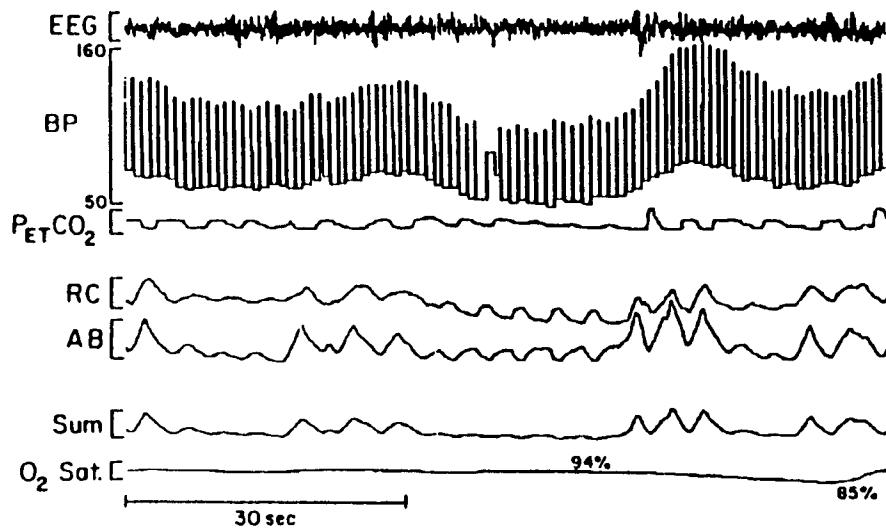


Figure 2 Population cohort subject with a moderate amount of undiagnosed sleep disordered breathing (apnea-hypopnea index=17/hr). Nasal CO_2 is used to estimate the presence or absence of airflow. RC, AB and SUM refer to measurements of rib cage and abdominal excursion estimating tidal volume. This phase of the record shows the following series: first a hypopnea, to mild hyperpnea to long apnea - most likely with airway closure (note paradoxical motion of RC in and AB out) - to hyperpnea to hypopnea. Blood pressure rises abruptly following each of the events, with or without cortical arousal.

timate the actual level of arterial pressure and left ventricular afterload existent over 24 hours. The decision threshold for diagnostic sleep studies should be lowered for the hypertensive patient. Thirdly, while most investigations focus on very severe forms of OSA, **more moderate levels of SDB**, such as recurrent hypopnea, and increased upper airway resistance, occur in a significant portion of the undiagnosed population.²⁶ These events also elicit acute pressor responses (Fig. 2) and contribute independently of other risk factors to relatively small but significant elevations in daytime blood pressure.²⁷ Accordingly, we need to define the levels of SDB that are truly "significant" pathophysiologically, and to determine the effects of early intervention. Finally, it is worth remembering that **hypertension is one of the strongest predictors of cardiovascular disease morbidity and mortality**. In light of the clear demonstration of a causal relationship between OSA and hypertension, accumulating evidence that cardiac failure, arrhythmias, stroke and myocardial infarction are more common in OSA patients, should receive more attention.

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CHAPTER 29

BASE HACE: AN INTRODUCTION

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Over the years I have grown a fondness for the brain at high altitude, but rather for the happy brain than the one that smarts because it is stressing the limits of its container. Nevertheless, today I have been given the assignment of beginning this session on the unhappy brain at high altitude, the one that goes under the acronym of HACE, High Altitude Cerebral Edema. My purpose will be to paint a clinical picture of what HACE is about: it's presentation, signs and symptoms, pathological picture, and treatment, and finally to set the stage for what will follow by Drs. Krasney and Severinghaus. The questions that will be explored by them this morning are: what causes the brain to swell and why do some succumb while others don't? For those of you who want to know more of the basics than I will have time or inclination to discuss here, I refer you to the fine review by Hamilton, et. al.¹³

A Bit Of The History

As Charlie Houston has taught me, the history of pain in the head on ascending to high altitude dates back to Chinese descriptions of the Greater and Lesser Headache Mountains written more than two millenia ago. These accounts differ from the more pleasant, euphoric inductions of hypoxia proffered by British and French balloonists of the late 19th century. In the instance of the flight of the Zenith from Paris in 1875, attempting a new altitude record with bladders of oxygen provided by Paul Bert, when the balloon returned to earth two of its three occupants were dead.¹ After an 1862 ascent to an altitude about that of Mount Everest, Sir James Glaisher described lying unable to move in the basket of the balloon, staring up at his companion:

I dimly saw Mr. Coxwell, and endeavored to speak, but could not. In an instant intense darkness overcame me, so that the optic nerve lost power suddenly, but I was still conscious, with as active a brain as whilst writing this. I thought I had been seized with asphyxia, and believed I should experience nothing more, as death would come unless we speedily descended: other thoughts were entering my mind when I suddenly became unconscious as on going to sleep.⁷

Probably the first modern description of what we now refer to as AMS was provided in 1913 by Dr. Thomas Ravenhill, a medical officer for mines in Chile.²¹ Although a surfeit of fluid in the lungs was first identified by Houston,¹⁴ and Hultgren¹⁷ in the early 1960s, the cerebral counterpart was a little slower to be

recognized, enabled by Hugo Chiodi's report of a case of severe symptoms of the cerebral form of high altitude illness,² Singh et al's observations reported in 1965²² and 1969,²³ which included neuropathological findings of brain swelling at post-mortem examination, and in 1975 a report by Houston and Dickinson of a dozen cases in Nepal.¹⁶

Signs and Symptoms

We now view HACE as different quantitatively, not qualitatively from AMS, Acute Mountain Sickness.^{10,16} AMS is customarily a transient affliction affecting mostly those ascending too high, too fast, but with curious differences in individual susceptibility. The most common symptom of AMS is headache, the discomfort of which helps define the severity of the condition. In addition, other symptoms include loss of appetite leading to nausea, then vomiting, lassitude and fatigue, malaise, dizziness, tinnitus, and difficulty sleeping; i.e. just feeling lousy. A variety of approaches to quantifying severity of AMS have been constructed, including the Environmental Symptom Questionnaire (ESQ) and the Lake Louise Score. AMS commonly lasts two to four days if the individual does not continue ascending. We have come to believe that the additional symptoms characterizing HACE simply represent increased severity of a common process that is dubbed AMS at its more benevolent end and cerebral edema when neurological abnormalities become more apparent (TABLE). As headache is to the diagnosis of AMS, ataxia is to defining HACE. Absent inebriation, a wobbling gait at altitude, likely cerebellar in origin, should be presumed due to HACE. Other signs and symptoms include being disoriented and confused, drowsiness, difficulty speaking, weakness or paralysis of arms or legs either one-sided or both, loss of the ability to stand, neck stiffness, nystagmus, and finally progressive loss of consciousness, which carries with it a poor prognosis for survival. Examination of the eyegrounds commonly reveals papilledema and often retinal hemorrhages, though the latter may be an independent phenomenon of high altitude, bearing no clear relationship to AMS or HACE.¹³

Table 1
HACE: Sign and Symptoms

- Severe headache
- Impaired motor and sensory function, especially ATAXIA
- Vomiting
- Impaired short term memory
- Papilledema
- Altered level of consciousness: disorientation to coma
- Hypo- or hypertonic reflexes
- Extensor plantar responses
- Hallucinations
- Visual: scotoma, field defects, blurred vision, pupillary changes
- Motor: tremor, difficulty speaking, cranial nerve palsies, rigidity or flaccidity, hemiparesis, meningismus, convulsions.
- Sensory: paresthesias, dysesthesias.
- Urinary incontinence.

The Epidemiology of HACE

HACE, being presumptively the more severe form of AMS, occurs under similar circumstances but with an incidence that may be as much as a couple orders of magnitude lower. Ascending too high, too fast is a trigger for both. HACE generally is not seen below 3600 m but has been reported to occur as low as 2500 m with rapid ascent.^{13,15} HACE also can strike even well-acclimatized individuals climbing at altitudes above 8000 m.³ The incidence, appreciably less than that of High Altitude Pulmonary Edema (HAPE), is not easy to pin down because population denominators have been hard to come by and because it is so altitude-dependent. Singh, et. al., enabled by the sudden transport of Indian soldiers to high altitude to defend their northern border, reported that between 3350 and 5500 meters the incidence of AMS was up to 8.3% with a HACE incidence of 1.2%.²³ At the elevation of ski areas in Summit County, Colorado (2400-3000 m), AMS, occasional cases of HAPE, and even more occasional cases of HACE, most commonly in individuals with HAPE, are seen among a large population ascending quickly from sea level. Younger individuals seem more susceptible to HACE but this difference could represent nothing more than a greater predilection and ability to put oneself in harm's way.⁹ Whether factors such as gender, hormonal changes, or ethnic differences influence susceptibility is not established. An association of a lower ventilatory response to hypoxia (HVR) with greater likelihood or severity of AMS has not been evaluated in regard to HACE. Mortality from HACE does occur, dependent in part upon how quickly treatment, particularly effecting conditions of a lower altitude, is implemented. HACE as a concomitant of HAPE is common, with a lesser likelihood of each occurring alone.⁴

Pathology

Modern imaging techniques have provided premortem access to diagnostic features as well as providing clues to the possible mechanisms causing HACE. MRI scans in particular have revealed white matter edema in T2-weighted scans, particularly in the region of the splenium of the corpus callosum.^{12,20} Additionally papilledema and increases in lumbar cerebrospinal fluid pressure support the contention of a swollen brain. Examination of the postmortem brain confirms the presence of white matter edema along with a diverse distribution of petechial hemorrhages (perivascular "ring and ball" appearance), blood-filled capillaries, and with associated but not certainly diagnostic hemorrhages in the cerebrum, upper brainstem and subarachnoid space.⁵ The intracranial hemorrhages are reminiscent of those seen in the retina and the extent to which they are an essential part of the picture seen with early HACE is not clear.

Prevention and Treatment of HACE

Ultimate prevention is afforded by avoiding ascent to high altitude in the first place, but lacking that, slower, staged ascent can go a long way toward decreasing the likelihood of AMS and its progression to HACE.⁹ Dexamethasone, a drug used commonly to treat other forms of cerebral edema, appears to have distinct value in both preventing and treating AMS/HACE,^{18,19} as do acetazolamide^{8,9} and possibly other diuretics.²³ Dexamethasone has both prophylactic and therapeutic efficacy in dealing with the cerebral symptoms of AMS and HACE.^{6,11} For prophylaxis it is best used with caution. It may be particularly useful when ascent has to be rapid

from sea level to above 4000 m, such as for rescue operations. For treatment its effect can be dramatic. The usual dose is 4 mg every 6 hours, perhaps with an initial 8 mg dose if acuity is great. Treatment is best accomplished by getting the sufferer to a physiologically kinder state of oxygenation, either by actual descent, by oxygen administration, or by relative hyperbaric therapy in something like a Gamow bag. Of these approaches, actual descent while the individual is still able to move under his or her own power, is most desirable (dexamethasone may be helpful to keep the afflicted individual ambulatory). No information yet exists identifying any difference in efficacy of the three approaches, presuming an equal elevation of arterial blood oxygenation with each technique. The decision as to which to choose is determined mainly by the situation.

The Mechanism Causing HACE and AMS: While the possibility that the symptoms of AMS result from a different process than the edema seen with HACE exists, current thinking is that the two represent simply different stages along a common spectrum.^{10,16} Although the possibility of a vascular cause for the headache exists, the finding that some of those with AMS but without clinical HACE exhibit MRI evidence of white matter edema and diminished ventricular size gives credence to a common process of intracranial fluid accumulation.^{12,20} While four of the seven individual who developed AMS when taken rapidly to 3700 m in the study by Matsuzawa et al had MRI findings of white matter edema, three with symptoms did not.²⁰ The correlation between ESQ score and MRI change appears quite strong, but whether those with increased white matter edema fell into the AMS domain or actually had mild HACE is not clear from the information provided. Even so, at this time, excess fluid in the brain, mainly in the white matter, seems the favored pathological correlate associated with and presumably causing the symptoms characterizing both HACE and its precursor, AMS.

If swelling is truly the underlying pathology, what causes the water to translocate into the tissues of the brain? Two, not necessarily mutually exclusive mechanisms have been proposed. One is that fluid enters the brain because of a breakdown in the integrity of brain blood vessels, referred to as vasogenic edema, which I interpret to mean a leak of vascular origin. The other is that hypoxia injures brain cells, perhaps both glia and neurons, causing intracellular accumulation of osmotically-active breakdown products that pull water into the brain, referred to as cytotoxic edema; this cause for edema is thought therefore to locate in the cellular regions of the brain, that is the gray matter. Years ago Houston and Dickinson proffered this latter mechanism,¹⁶ but the recent MRI observations that the edema is concentrated in white rather than gray matter have focused attention on a vasogenic origin.¹² For one thing, there is no evidence that the sodium-potassium pump failure that might trigger calcium release and cell injury occurs at what in fact can be a rather moderate level of hypoxia. If the problem is one of vascular leak, then what causes the leak? Is it a mechanical phenomenon resulting from flow, pressure and stretch of the endothelium of the blood-brain barrier, perhaps breaking down the tight junctions joining endothelial cells? Or is endothelial cell permeability increased as a consequence of activation and release of chemical mediators that have the ability to damage brain capillary endothelium and/or its tight junctions?

Having posed these questions, I will pass the baton of inquiry to my erudite successors on this program to share their thoughts on how a vascular leak as the

cause of HACE (and AMS) might come about and why some of us are more likely to get brain swelling than others. Realize, though, that what follows falls within the scientific domain referred to as hypothesis, meaning to a lad from Missouri "show me".

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CHAPTER 30

CEREBRAL HEMODYNAMICS AND HIGH ALTITUDE CEREBRAL EDEMA

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It is now generally accepted that acute mountain sickness (AMS) is caused by a mild form of high altitude cerebral edema (HACE). AMS may progress to a potentially lethal form of HACE or high altitude pulmonary edema (HAPE). Systemic manifestations of AMS such as fluid retention and peripheral edema may be linked to HACE.²¹ HACE may also occur in patients experiencing chronic hypoxia. Although there have been several excellent reviews of the altitude illnesses, most hypotheses as to the etiology of these disorders have been based on anecdotal evidence.^{7,10,19} Several years ago, our laboratory developed and standardized a conscious instrumented sheep model for the study of AMS-HACE.^{5,21,32}

AMS can lead to HACE and/or HAPE along with significant peripheral edema²¹ suggesting a common mechanism. Hansen and Evans first reviewed evidence in 1970 that high altitude is associated with a shift of fluid into the brain, elevated cerebrospinal fluid (CSF) pressure and elevated cerebral blood flow (CBF). They suggested that AMS results from compression of brain cells.⁸ Lassen and Harper²² and Sutton and Lassen²⁷ proposed that elevated brain capillary hydrostatic pressure (Pcap) secondary to hypoxic vasodilation causes increased capillary filtration and permeability similar to the vascular "autoregulatory breakthrough" which occurs in hypertensive encephalopathy.¹⁸ This is termed the "vasogenic edema hypothesis". By contrast, Houston and Dickinson¹¹ postulated that AMS-HACE is due to failure of the Na/K pumps in brain cell membranes due to hypoxia. Thus, brain swelling at altitude may be attributed to failure of cell volume regulation and/or depression of ion pumping at the blood-brain barrier (BBB). This is termed the "cytotoxic edema hypothesis".

Our data indicate that awake sheep exposed to normobaric arterial hypoxia for 4-5 d with an arterial PO₂ of 40 torr develop clinical signs of AMS. This represents an arterial O₂ saturation of ~50% although in a human the O₂ saturation would be ~75% at this PO₂. AMS is judged to be present when food and water intake decline to ~30% of pre-hypoxic levels³² and by increased lethargy and frequency of lying down. Curran-Everett et al⁵ found that 14/22 sheep exposed to hypocapnic hypoxia

Table 1

Wet/dry ratios and percent brain water in sheep exposed to sustained hypoxia.
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	<i>n</i>	W/D Ratios	% Brain Water
Frontal lobe			
Control	7	4.939±0.023	79.75±0.09
Non-AMS	3	5.026±0.034	80.10±0.11
HH	6	5.209±0.032*	80.80±0.10*
EH	9	5.155±0.029*	80.60±0.10*
Occipital lobe			
Control	7	4.956±0.033	79.82±0.13
Non-AMS	3	5.024±0.046	80.09±0.14
HH	6	5.264±0.054*	80.99±0.18*
EH	9	5.183±0.030*	80.70±0.11*

Values are means \pm SE; *n*, no. of sheep. W/D, wet-to-dry weight; % brain water = (wet tissue wt — dry tissue wt)/wet tissue wt. * Significantly different from corresponding control and non-AMS values ($P<0.05$).

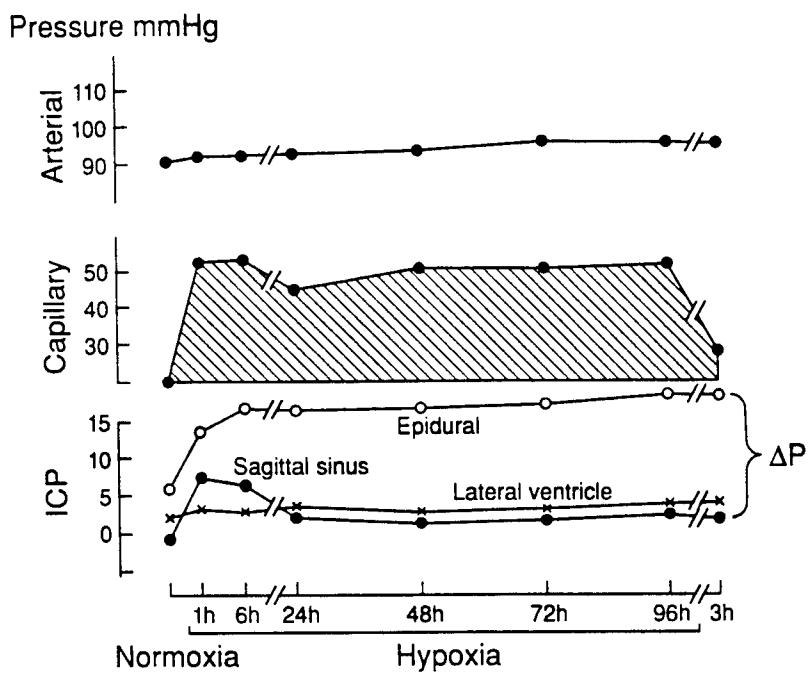


Figure 1 Intracranial (ICP), cerebral capillary, and mean arterial pressures in AMS sheep during 5 days of normobaric hypoxic hypoxia. See text for explanation. Reproduced by permission of The American College of Sports Medicine.

(HH) developed AMS while Yang et al³² found AMS occurring in 9/12 animals exposed to HH. This incidence is similar to that in humans.²¹ When sheep were exposed to eucapnic hypoxia (EH), all animals developed AMS.³² Curran-Everett et

al⁵ found increased brain water primarily in white matter in AMS sheep. Table 1 indicates that, although non-AMS sheep exhibit small increases in brain water, significant increases are demonstrated only in AMS sheep exposed to either HH or EH.³² Similar results were obtained in a study by Yang et al³⁴ in Chungking where all goats exposed to hypobaric HH had increases in brain water.

Figure 1 represents data from AMS sheep consolidated from several studies²¹ which indicate that estimated brain Pcap is elevated during HH over 96 h along with epidural pressure which is the most accurate indicator of brain tissue pressure. By contrast, sagittal sinus pressure or cerebral venous pressure (Pv) only rises temporarily and lateral ventricle pressure fails to change. The later decline of Pv may reflect collapse of veins draining into the sagittal sinus due to brain swelling. It is evident that a pressure gradient develops between the epidural space and the venous compartment which favors reabsorption of fluid by the arachnoid villi. Arterial pressure does not change. The elevation of epidural pressure does not occur in non-AMS sheep. At autopsy, the brains of the AMS-HACE sheep are found to be turgid with congested pial vessels and multiple petechial hemorrhages similar to humans with HACE.²³ After 24 h, it is difficult to withdraw CSF from a lateral ventricle catheter in AMS-HACE sheep. In neurological terms, the edema is quite severe and is not readily reversible by restoration to room air.

AMS-HACE is associated with a major transcapillary shift of fluid into the brain as shown in Figure 2. Iwamoto et al¹⁴ studied cerebral vascular responses during the first several hours of HH and found that arterial and cerebral venous hematocrit and hemoglobin levels rise with CBF (radiolabeled microspheres) for the initial 90-120 min. Subsequently, cerebral venous hematocrit and hemoglobin levels rise well above arterial levels indicating cerebral venous hemoconcentration. These data indicate that the magnitude of the fluid shift is quite large and raise questions as to how the brain accommodates the entry of this volume of fluid. It is also unclear as to why ~2 h are required for the fluid shift to develop. It is possible that, after 2 h of vasodilation, structural forces are overcome and flooding of the extracellular spaces occurs.²¹ Our observations indicate that permeability of the BBB to protein is also increased in sustained hypoxia as judged by patchy staining of brain tissue with 2% Evans blue.¹

Figure 3 indicates that regional CBF is increased in sustained fashion during both HH and EH with increases in cortical and brainstem regions being comparable. The flow increases appear to be uniform with no areas of underperfusion as estimated by radiolabeled microspheres. It is evident that CBF increases more with EH than during HH and there is a decline in choroid plexus flow during HH but not during EH. The latter may reflect a decline in formation of CSF during HH. As the result of these CBF responses, brain O₂ and glucose deliveries are either maintained constant (HH) or are increased (EH) (Fig. 4) to the extent that cerebral fractional extractions of O₂ and glucose are either unchanged (HH) or decreased (EH) (Fig. 5). Brain O₂ uptake was unchanged in either HH or EH (Fig. 6) while brain glucose uptake rose transiently only at the onset of HH. Brain lactate output rose only at the onset of HH as did the glucose oxygen index. Thus, there are no deficits in brain O₂ or glucose delivery during sustained hypoxia. Brain O₂ and glucose deliveries were in excess of metabolic demand during EH, yet all animals became ill. The temporary elevation of brain lactate output in HH may reflect activation of phosphofructokinase secondary to a temporary imbalance between production and utilization of

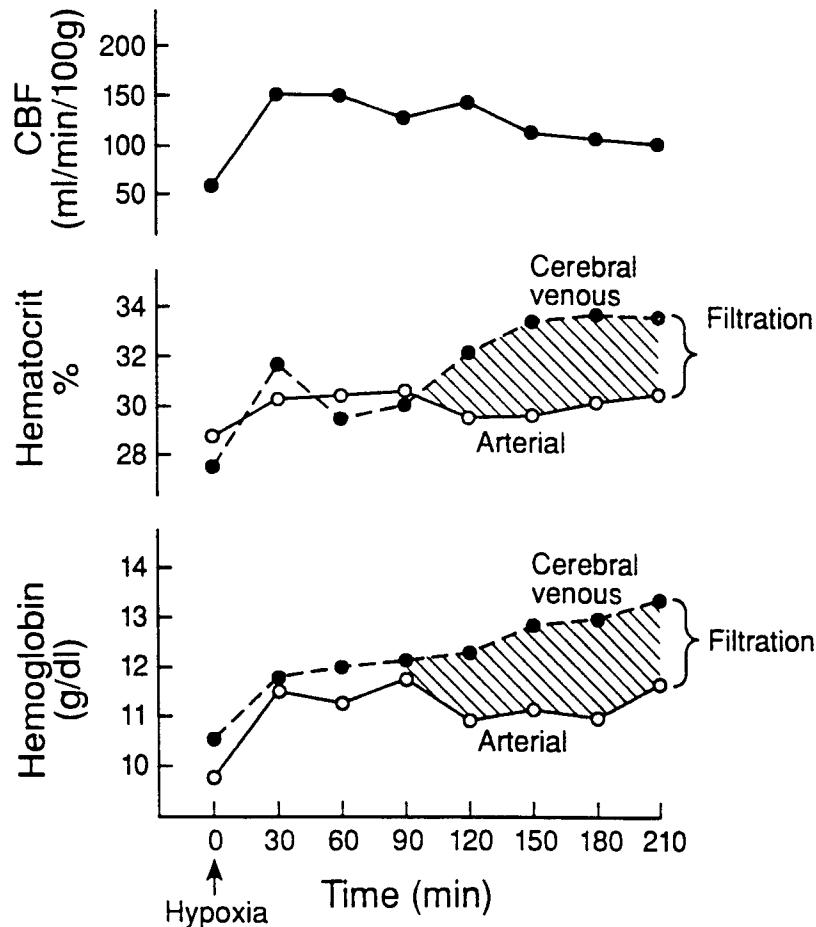


Figure 2 Cerebral blood flow (CBF), systemic arterial and cerebral venous hematocrit and hemoglobin responses in sheep during 3 h of hypoxic hypoxia. See text for explanation. Reproduced by permission of the American Physiological Society.

high-energy phosphates. Phosphofructokinase is pH sensitive. Therefore, the blood alkalosis may be also in part responsible for the increase in glucose uptake and glucose-oxygen index early in HH. This imbalance is likely rapidly corrected by the rise of CBF. Lactate output and glucose uptake were unchanged during EH, probably due to the higher CBF. Thus, there is little evidence that brain energetics are altered during sustained hypoxia.³² Moreover, these data suggest there is little relation between either CBF or delivery of substrate to the brain and the incidence of AMS-HACE. Jensen et al¹⁷ also were unable to demonstrate a relation between the magnitude of the CBF response and incidence of AMS in humans using ¹³³ Xenon. By contrast, Baumgartner et al³ reported that subjects exhibiting AMS had higher levels of CBF as estimated by transcranial doppler. However, the subjects with AMS were also more hypoxic.

We carried out two additional studies in awake sheep to determine whether sustained cerebral vasodilation *per se* in the absence of hypoxia can elicit increases in

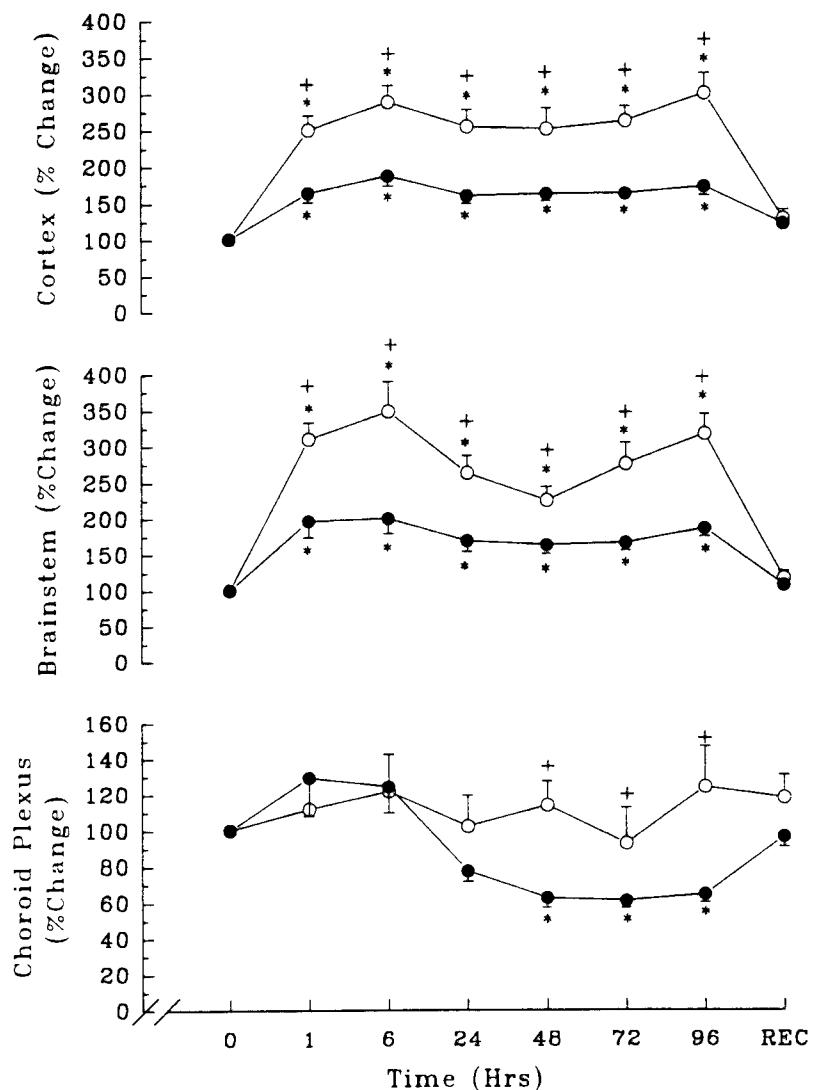


Figure 3 Regional CBF responses (radiolabeled microspheres) in awake sheep during 5 days of hypoxia. Closed circles, sheep exposed to hypocapnic hypoxia. Open circles, sheep exposed to eucapnic hypoxia. REC, 3 h after restoration to room air. % change from control (± 1 SE). * = $p < 0.05$ from control, + = $p < 0.05$ from corresponding hypocapnic value. See text for explanation. Reproduced by permission of the American College of Sports Medicine.

brain water and AMS-HACE-like responses. Figure 7 indicates the CBF responses occurring during exposure of sheep to 5% CO₂ in air for 96 h. In spite of larger increases in CBF (and presumably Pcap) than those occurring in hypoxia, these sheep continued to eat and drink and exhibited no signs of AMS for the entire period. However, we were able to detect small increases in brain water which were significantly less however than those observed in AMS-HACE. Figure 8 shows the

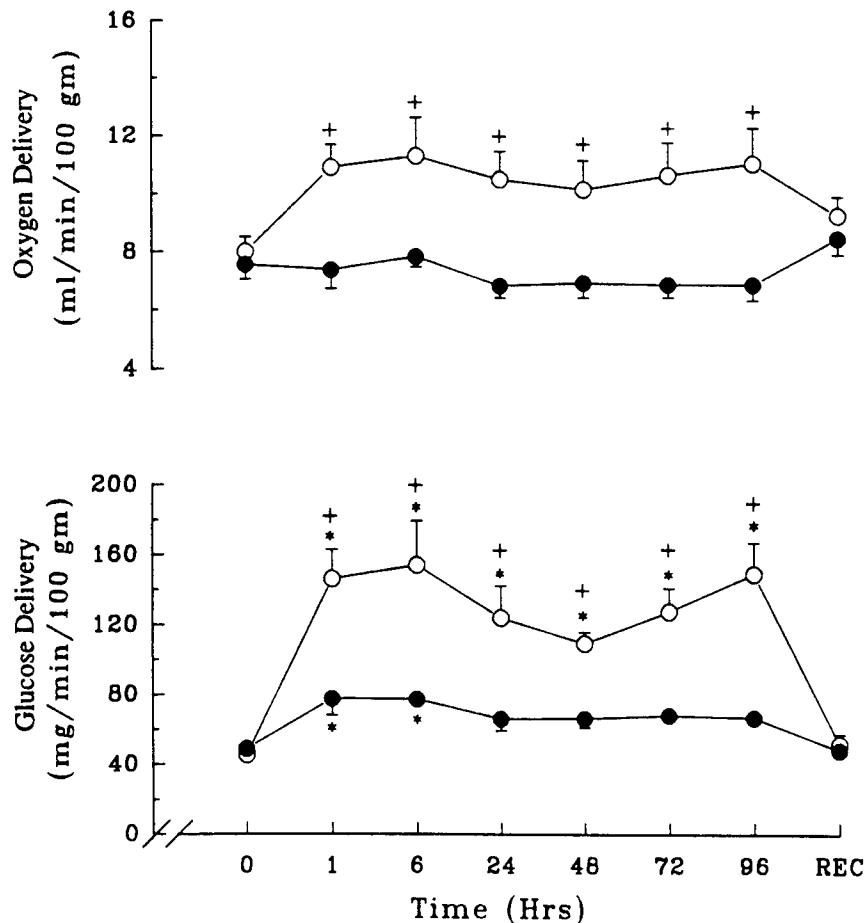


Figure 4 Cerebral oxygen delivery (upper panel) and glucose delivery (lower panel) in hypocapnic hypoxic (closed circles) and eucapnic hypoxic (open circles) sheep. * $=p<0.05$ from control value, + $=p<0.05$ from corresponding hypocapnic value. See text for explanation. Reproduced by permission of the American Physiological Society.

effect of 4 h infusions of nitroglycerin on CBF. CBF was elevated significantly both during the infusion and after the infusion. However, in spite of the sustained vasodilation for a time period during which, in hypoxia would usually be associated with a major fluid shift, these animals continued to eat and drink, failed to display signs of AMS and brain water content did not increase.

Possible Mechanisms of HACE: Our conscious sheep data indicate that there is no alteration in brain energetics for oxygen or glucose in animals developing severe AMS-HACE with the exception of an early transient elevation of brain glucose uptake and lactate output in animals exposed to HH. As judged by decreases in cerebral fractional extractions of oxygen and glucose in EH, the deliveries of oxygen and glucose were in excess of normal, yet all the EH animals became ill. These observations render it difficult to assign an important role for cytotoxic mechanisms^{10,11} in AMS-HACE, and suggest that hypoxic failure of either intraparenchymal

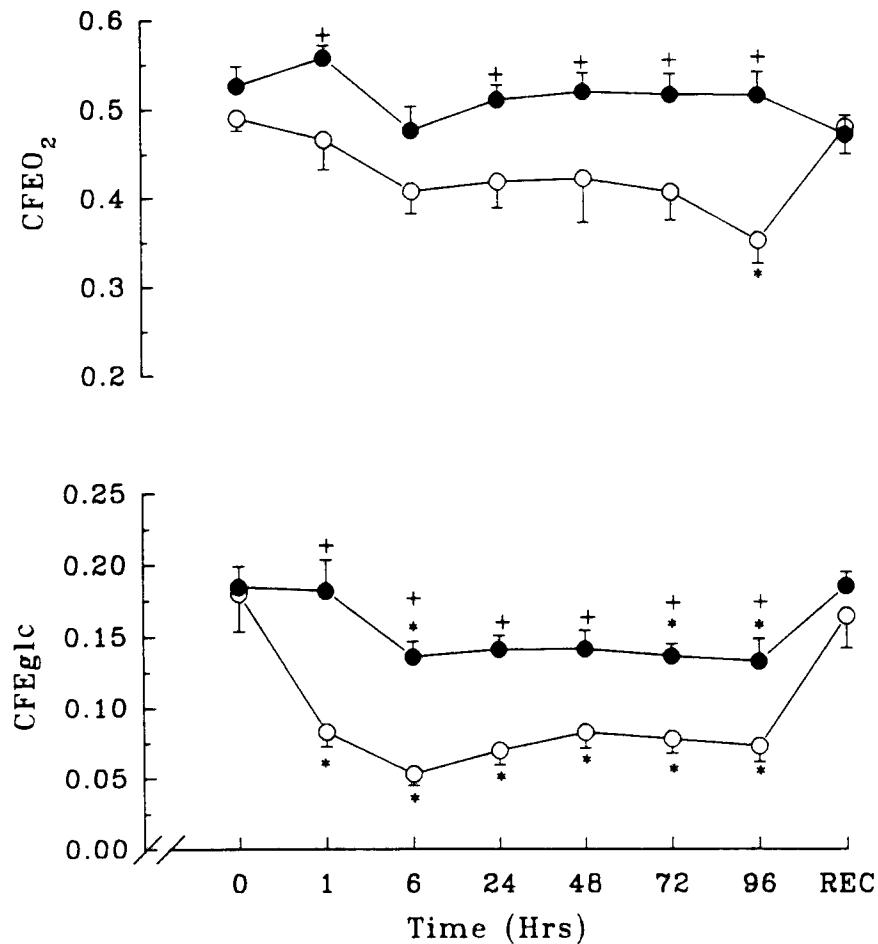


Figure 5 Cerebral fractional extractions of oxygen (CFE_{O₂}) and glucose (CFE_{glc}) in HH and EH sheep. Abbreviations as in Figure 4. See text for explanation. Reproduced by permission of the American Physiological Society.

mal cell volume regulation or BBB endothelial cell (EC) ion transport is unlikely. It is important to recognize however that specific studies to determine whether brain cell volume changes in sustained hypoxia and AMS-HACE have not been performed.

Since deliveries of oxygen and glucose are unchanged (HH) or even increased (EH) during sustained hypoxia, important questions remain as to the nature of the sensor and of the signal which mediates cerebral vasodilation during hypoxia. It seems very likely that adenosine is an important initiator of the brain vasodilator response to hypoxia³¹ and that the vasodilator response is sustained and modulated by release of nitric oxide (NO) via shear stress on vascular EC (flow-mediated vasodilation)^{13,15,16}.

The vasogenic edema hypothesis implies that flooding of the extracellular spaces occurs secondary to stress failure of cerebral capillaries similar to that postulated for

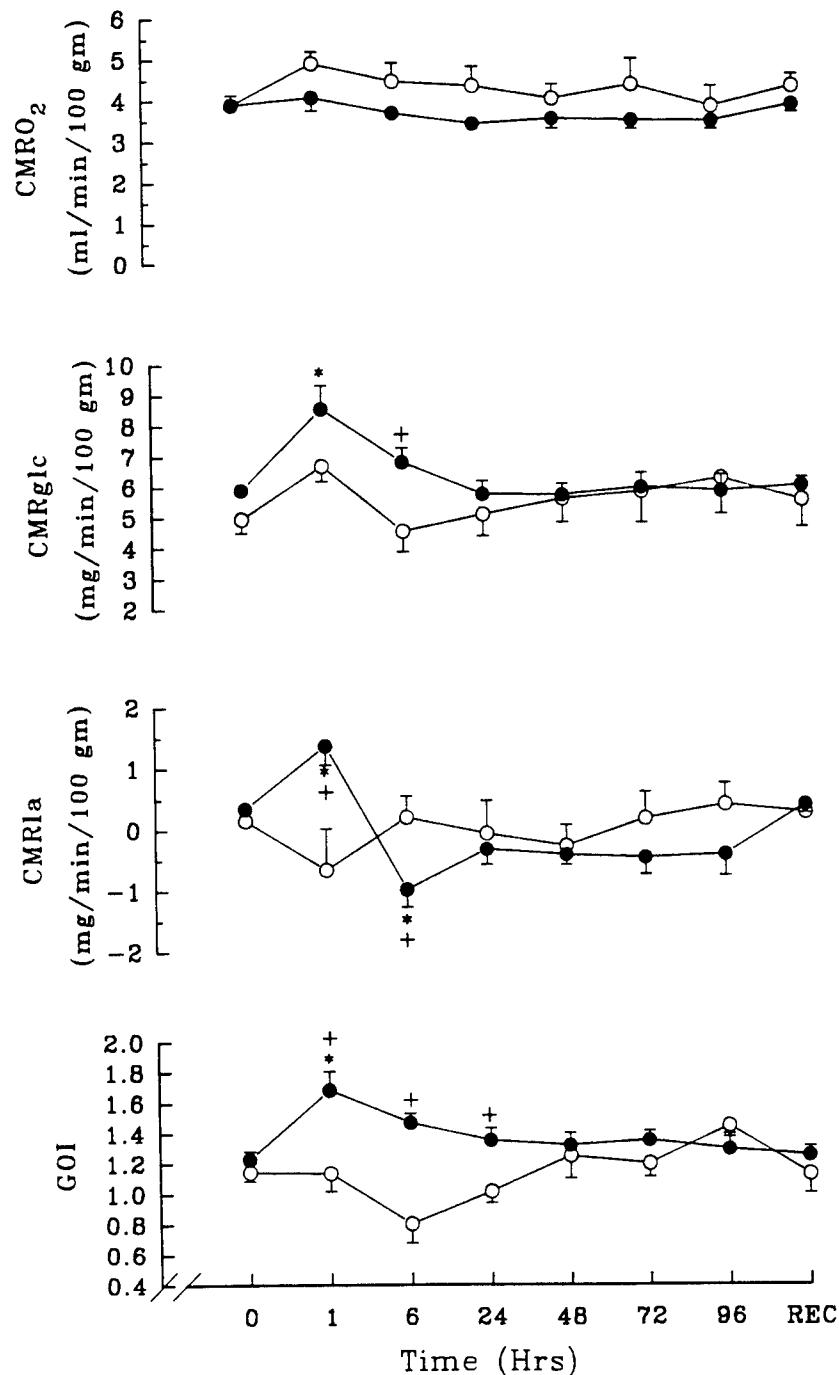


Figure 6 Cerebral metabolic rates of oxygen (CMRO₂) and glucose (CMR_{glc}), lactate release (CMR_{lac}) and the glucose-oxygen index (GOI) in HH and EH sheep. Abbreviations as in Figure 4. See text for explanation. Reproduced by permission of the American Physiological Society.

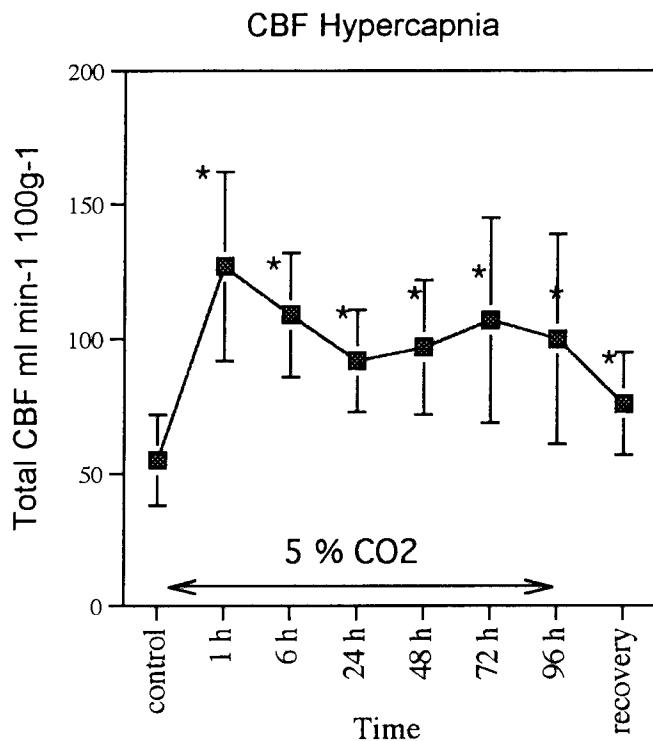


Figure 7 CBF responses (radiolabeled microspheres) in 8 awake sheep exposed to hypercapnia for 96 h. Recovery values obtained 3 h after return to room air. Values are means \pm 1 SD, * $=$ p<0.05 relative to pre-hypercapnia value. Despite CBF responses greater than those elicited by hypoxia, sheep fail to develop AMS-like signs. Data plotted from reference 33.

the pulmonary circulation.³⁰ Since the BBB is located in EC tight junctions the hypothesis is that Pcap ruptures the tight junctions if the hydrostatic pressure is of sufficient magnitude or applied for a long enough period of time. The data clearly indicate that elevations of CBF and Pcap occur during sustained hypoxia. However, while these responses occur in all animals exposed to hypoxia, not all animals develop AMS-HACE. In addition, sustained elevations of CBF in non-hypoxic circumstances such as hypercapnia or infusion of nitroglycerin fail to elicit AMS-HACE-like responses. These observations suggest that the elevations of Pcap which are elicited during sustained hypoxia are of insufficient magnitude to open EC tight junctions.

It is a classic observation that acute hypertension with elevations of arterial pressure on the order of +60 mm Hg open the BBB very rapidly^{18,27} Mayhan and Heistad²⁴ measured pial venous pressure increments of about +24 mm Hg when systemic arterial pressure was increased by +60 mm Hg using a servo-null method in the rat. By comparison, sagittal sinus or cerebral venous pressure only rises by 8 to 10 mm Hg temporarily in awake sheep during sustained hypoxia and arterial pressure does not change.^{14,32} Mayhan and Heistad²⁴ reported that acute hypertension causes pial venules to begin leaking within seconds. By contrast, the cerebral tran-

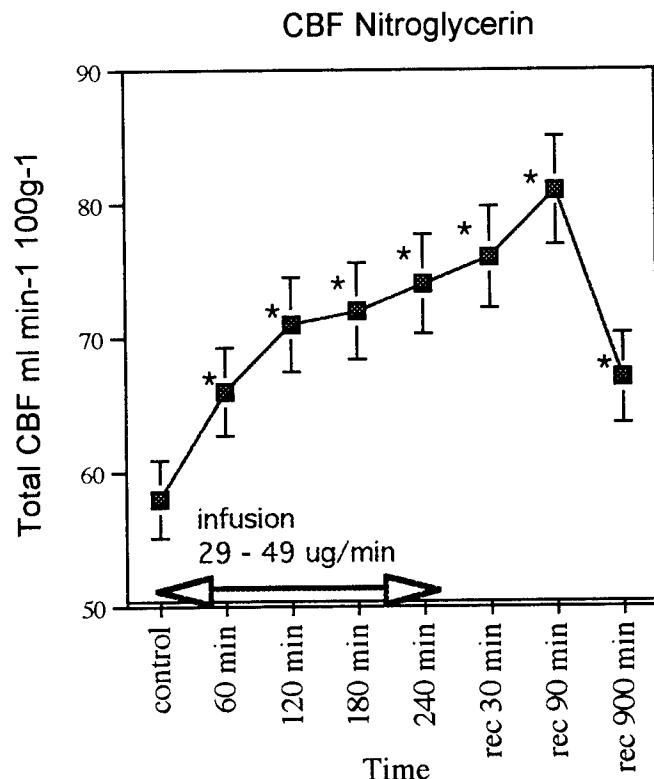


Figure 8 CBF responses (radiolabeled microspheres) to infusion of nitroglycerin for 4 h in 9 awake sheep. Values are means \pm 1SD, * $=$ p<0.05 relative to pre-infusion value. Despite sustained vasodilation persisting for 15 h after stopping the infusion sheep fail to develop AMS-like signs.

scapillary fluid shift during hypoxia requires 90-120 min to become evident and the vascular lesions of AMS-HACE are characterized by multiple petechial hemorrhages. Thus, available data suggest that the elevation of Pcap in hypoxia is, by itself, of insufficient magnitude to open the BBB. However, once the BBB is opened, the elevated Pcap very likely promotes the transvascular movement of fluid and macromolecules.

Autoregulation is defined as the tendency for an organ or tissue to maintain blood flow relatively constant during changes in arterial perfusion pressure. The autoregulatory mechanism is well-developed in the cerebral circuit and serves to protect the brain microcirculation from upstream pressure surges. Unfortunately, there is little data as to the specific effect of hypoxia on the cerebral autoregulatory mechanism. Relaxation of cerebral vascular arterioles during hypoxia could abolish autoregulation such that flow becomes passively related to the pressure gradient. Alternatively, the presence of a vasodilator stimulus may cause the autoregulatory mechanism to be "reset" such that flow is regulated at a higher level during changes in perfusion pressure (Fig. 9). Several studies suggest that activation of the sympathetic innervation of cerebral vessels protects the microcirculation by modulating

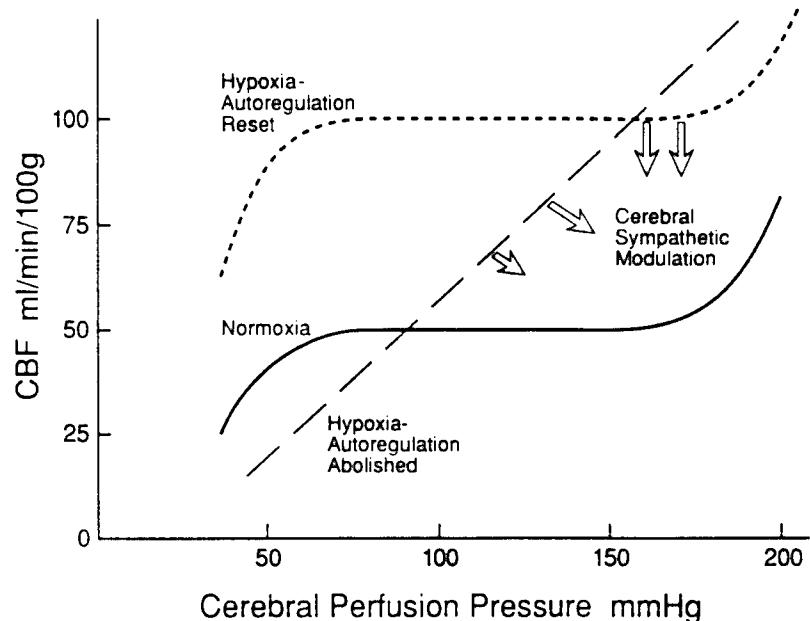


Figure 9 Cerebral autoregulation of blood flow during sustained hypoxia: If autoregulation is abolished, CBF would passively follow changes in perfusion pressure. Autoregulation may be reset such that CBF is higher for a given perfusion pressure. Activation of cerebral sympathetics may minimize increases in CBF and protect the microcirculation. Reproduced by permission of the American College of Sports Medicine.

elevations in capillary pressure during acute hypoxia.^{4,20,29} There is a need to determine whether sympathetic modulation of brain microvascular pressures is important in the etiology of HACE (Fig. 9). If either autoregulation and/or sympathetic modulation of microvascular pressure are impaired during hypoxia, it is quite possible that elevations of systemic pressure evinced during isometric exercise maneuvers for example, could be propagated to the microcirculation raising Pcap temporarily to a level sufficient to open EC tight junctions.

The observations described thus far lead to the conclusion that, with the exception of maneuvers that elicit excessive elevations of systemic arterial pressure, elevations of Pcap during sustained hypoxia are not large enough to open the BBB. This suggests that some other mechanism or mechanisms must be activated simultaneously to increase the permeability of the BBB. It has been demonstrated that acute hypoxia is associated with a systemic increase in capillary permeability as judged by increased transcapillary escape of albumin.⁹ The extent to which this increase in permeability occurs in the cerebral circuit is unclear. It is likely that this generalized increase in capillary permeability is in part secondary to the decline of cyclic adenosine monophosphate (cAMP) levels which is observed in cultured endothelial cell monolayers exposed to acute hypoxia. In association with the decline in cAMP levels there is an increase in the permeability of EC monolayers to several macromolecules, including albumin, in hypoxia.²⁵ The decline of EC cAMP levels and the increased EC permeability during acute hypoxia are offset by dexamethasone in a

dose-dependent manner.²⁵ The role of the decline of EC cAMP levels in the etiology of AMS-HACE remains to be determined.

In addition to cAMP, it is possible that cyclic guanosine monophosphate (cGMP) modulates BBB permeability in hypoxia. Although the data are somewhat inconsistent, several studies suggest that nitric oxide and cGMP act to sustain or maintain permeability of the BBB. Inhibition of NOS appears to open the BBB and is associated with leakage of horseradish peroxidase into the extravascular space.²⁶ NO is released from cerebral non-adrenergic, non-cholinergic nerves (nitroxidergic nerves) and causes cerebral vasodilation.¹² Regional nitroxidergic vasodilation may be part of the brain hypoxic vasodilator response.^{13,15,16} In addition, NO is released by EC shear stress and can mediate flow-mediated vasodilation during hypoxia.¹³ Thus, NO released either from nerves or via shear stress could modulate BBB permeability via its effect on cGMP. We have shown that NO is important for supporting brain metabolism and consciousness during hypoxia in the awake sheep.¹⁶

There is a growing body of evidence which suggests that activation of leukocytes may play a key role in the etiology of several microvascular pathophysiological responses. Schmid-Schonbein et al²⁸ have postulated that the increased risk of cerebral vascular accident in essential hypertension is secondary to activation of circulating neutrophils which adhere to the vascular endothelium in the brain. Circulating levels of activated leukocytes are significantly elevated in spontaneously hypertensive rats and in essential hypertension in humans.²⁸ Activated leukocytes adhering to the vascular wall can release cytokines and initiate a host of responses which can lead to opening of EC tight junctions. In addition to hypertension, there is evidence that activation of leukocytes plays an important role in increasing microvascular permeability in hemorrhage.² Elevation of systemic catecholamine levels leads to activation of neutrophils and increased adhesion energy. Since there is ample evidence for elevation of circulating catecholamine levels in hypoxia, it is very likely that levels of circulating activated neutrophils are elevated as well. Cerebral hemodynamic behavior may favor local adhesion of leukocytes along with the activation process. Histological sections from our sick sheep indicate local infiltration of neutrophils in the petechial hemorrhagic lesions. Thus, HACE may be a manifestation of a systemic inflammatory response. This is in keeping with the fact that several hours are usually required for AMS-HACE to become manifest. Further studies of the *in vivo* behavior of leukocytes in the systemic circulation and the brain microcirculation during hypoxia are warranted.

In summary, our sheep model of AMS-HACE suggests that cerebral energetics are unaltered during sustained hypoxia implying that cytotoxic swelling of cells is unlikely to be the cause of HACE. It is rather more likely that HACE represents vasogenic edema secondary to a transcapillary shift of fluid and macromolecules. However, our results suggest that, although cerebral capillary hydrostatic pressure is elevated during hypoxia, the magnitude of the rise in capillary pressure by itself is insufficient to open endothelial cell tight junctions which constitute the blood-brain barrier. Sustained cerebral vasodilation occurring in the non-hypoxic situations of hypercapnia or nitroglycerin infusion does not elicit AMS-HACE-like responses. However, failure of protective mechanisms such as autoregulation or cerebral sympathetic vasoconstriction could allow cerebral microvascular pressure to rise high enough to open the blood-brain barrier. Current results point to some other mechanism or mechanisms which operate(s) in concert with the elevated capillary

hydrostatic pressure to increase blood-brain barrier permeability. Both cAMP and NO-cGMP- related mechanisms appear to be important modulators of microvascular permeability during hypoxia and the relative importance of these mechanisms in the etiology of AMS-HACE requires clarification. Several observations suggest that hypoxia is likely to be associated with elevated levels of circulating activated leukocytes which may adhere to the cerebral vascular endothelium and provoke leaks of the blood-brain barrier.

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CHAPTER 31

ETIOLOGY OF HACE: HYPOTHESES AND STUDIES

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In 1995, I suggested three possible etiologies of HACE: angiogenesis, osmotic swelling, and ischemia.¹ Here I propose to briefly review the ideas underlying the latter two, propose one more wild but easily tested idea, and present research on the most potent angiogenesis cytokine, VEGF, conducted by Dr. Feng Ping Xu during his year with me at UCSF.

1) **Osmotic swelling:** In hypoxia, the mechanisms which keep V_o_2 constant cause cells to swell. Two interdependent, synergistic "laws" explain this: 1) The Pasteur effect (ADP control of glycolysis); and 2) The law of mass action at the aa₃ cytochrome oxidase: $V_o_2 = \dot{e} \cdot P_o_2$. Hypoxia alters the mass balance, causing electrons to accumulate (redox reduction) until the product of electron pressure (voltage) and P_o_2 is constant. A transient fall of V_o_2 causes ADP to rise. Increased glycolysis raises intracellular concentrations of all substrates (pyruvate, lactate), and all the intra-mitochondrial citric acid cycle intermediates and reducing equivalents enough to restore a new mass balance, restoring a constant V_o_2 . Steady state V_o_2 remains constant until P_o_2 at the aa₃ metal surface has fallen to nearly 0, when aa₃ becomes as much as 99.5% reduced. At this point, the rising substrate concentrations have increased osmotic pressure by about 30mOsm, causing about 10% swelling. If O₂ delivery falls even more, V_o_2 falls. After 3 min. of anoxia or circulatory arrest, brain cells take up almost all ECF water. If flow of hypoxic blood continues, water and glucose continue to move into cells, and swelling is greater. This mechanism may contribute to the prompt headache of severe hypoxia in AMS, and might be part of HACE.

2) **Ischemia:** As cells swell in the non-expandable calvarium, after CSF is displaced, blood will be also displaced by compression, and this may render some vulnerable areas more ischemic, thereby further promoting osmotic swelling.

3) **Bubbles:** A new hypothesis worth testing is an altitude form of bends: At sea level, tissue and venous total gas pressure is 50 torr lower than arterial and ambient gas pressure because, as blood traverses tissue, P_o_2 falls from 100 to 40 torr while P_{CO_2} rises only about 10 torr. Venous blood is thus able to take up N₂ from bubbles. At 5km altitude, PaO_2 falls to the steep part of the O₂ dissociation curve. P_o_2 in venous blood and tissue may be only about 8 torr lower than PaO_2 ; tissue P_{CO_2} is about 8 torr higher than $PaCO_2$. Tissue gas pressure is thus equal to ambient pressure. Bubbles, if formed, become stable because N₂ cannot diffuse out to blood. When cerebral blood flow is stopped experimentally, brain P_{CO_2} rises to 250-400 torr. Hypoxic lactic acid titration of tissue HCO₃⁻ stores may generate high enough tissue P_{CO_2} to initiate bubble formation. It requires only brief ischemia to raise tis-

sue gas pressure well above the low ambient pressure at extreme altitude. Bubble formation usually does not occur easily unless a nidus exists and a low surface tension can be provided. Perhaps some cells contain these essentials. There are reasons to suspect that some intracellular vesicles normally contain gas.

This hypothesis can easily be tested by recording the effect of breathing 80% N₂O for 5 min on CSF pressure in dogs after a few hours in an altitude chamber, say at 5.5 Km. Breathing 80% N₂O increases the size of any gas space by nearly 5 fold within minutes. (At Pb=390 torr, 80% N₂O is sub-anesthetic). After a few minutes of breathing 100% O₂ to eliminate bubbles, N₂O should have much less effect on ICP.

4) **Angiogenesis**, studies conducted by F. P. Xu:

Tissue hypoxia initiates angiogenesis by expressing vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF). This cytokine dissolves basement membranes of capillaries, permitting endothelial budding and neovascularization. This results in capillary leakage of plasma and red cells.² The permeability enhancing effect of VEGF is 50,000 times more potent than histamine. VEGF stimulates von Willebrand factor expression by endothelial cells and induces thromboplastin activity.³ These effects suggested that VEGF might be the culprit causing the formation of cerebral thrombosis and petechial hemorrhages in HACE.

Xu determined the time course of brain VEGF response to systemic hypoxia. In a pilot experiment he found rabbits could survive at least 14 days with as low as 6% oxygen. However, rabbits seemed to be less tolerant of hypoxia than rats. During exposure, 6 out of 16 rabbits died while only 2 out of 34 rats died. All deaths occurred during the first three days of hypoxia. Brain surface vascularity appeared more congested in the hypoxic animals than in controls. Histopathologic examination of brain from one rabbit after 6 days in hypoxia revealed petechial hemorrhages throughout the brain.

Xu has now assessed brain VEGF expression in 32 rats exposed to increasingly severe normobaric hypoxia (9% to 6% O₂) for 3, 6 or 12 hours, or 1, 2, 3 or 6 days. O₂ concentration was repeatedly titrated to maintain limited activity and decreased intake of food and water. During the first 3 days the tolerable level of O₂ appeared to stabilize or, in some cases, rose.

Northern blot analysis demonstrated that 2 molecular bands of transcribed VEGF mRNA, approximately 3.9 kb and 4.7 kb, were up-regulated in cortex and cerebellum after as little as 3 hours of hypoxia peaking at 12-24 hours, but subsequently falling despite constant or increasingly severe hypoxia. Western blot revealed that VEGF protein was increased after 12 hours of hypoxia reaching a maximum in about 2 days. Enhanced VEGF mRNA persisted for at least 6 days. Cortex was not significantly different from cerebellum, as assessed by western blot. He also found a 16 hr. peak in rabbit brain VEGF mRNA which then declined for the next 2 weeks of increasingly severe hypoxia.

VEGF may exist in four different homodimeric molecular species due to alternative splicing of mRNA, with 121, 165, 189, or 206 amino acids. The two VEGF mRNA transcriptions, of 3.9 kb and 4.7 kb, were increased in hypoxic brain at 24 hrs. These are believed to represent the isoforms with 165 and 189 amino acids, respectively. Western blot analysis of rat brain lysates resolved under reducing conditions showed a VEGF band at 23kDa, consistent with the monomer of VEGF₁₆₅. VEGF₁₆₅ is the most abundant molecular species in all human tissues

except placenta. The VEGF isoforms have different properties *in vitro*, which may determine their function *in vivo*. The two shorter forms, VEGF₁₂₁ and VEGF₁₆₅, are secreted and likely to be available in physiological conditions, the longer ones, VEGF₁₈₉ and VEGF₂₀₆, are bound to heparin-containing proteoglycans in the cell surface or in the basement membrane. All four molecular species of VEGF can promote dye extravasation when applied in a guinea pig skin permeability assay.

Xu found that VEGF was expressed abundantly in normal lung tissue but was not obviously upregulated within 24 hours of hypoxia.

VEGF is said to bind with high affinity to endothelial cells in arteries, veins and microvessels through its two tyrosine kinase receptors: the fms-like tyrosine kinase receptor (flt-1) and the tyrosine kinase receptor (KDR).⁴ Flt-1 mRNA in brain tissue of both rabbits and rats was induced after 2-3 days of hypoxia, supporting the notion that VEGF is acting on brain vessels. The binding sites of iodinated recombinant human VEGF (¹²⁵I rh-VEGF) are distributed throughout all the tissues of the adult rat in a pattern that reflects the distinct vascularization of each organ, with the highest binding level in brain, adrenal cortex, glandular stomach, lung, spleen and pancreas. In the central nervous system, the density of binding was greatest in gray matter. In the cortex, the binding pattern following the distribution of penetrating vessels extending from the outer meninges toward the deeper laminae. Displaceable binding was also associated with the meninges and the large vessels on the surface of the brain and seen along vascular elements.⁵

Recently, Liu et al have identified a hypoxia-responsive enhancer in the promoter region of human VEGF gene, which includes a 28-bp element that mediates the upregulation of VEGF gene transcription. Hypoxia inducible factor -1 (HIF-1) has been shown to bind to this element.⁶ Post-transcriptional mechanisms have also been suggested as facilitating hypoxic induction of VEGF.

The implication of these animal studies that VEGF may act as a mediator in the process of hypoxic cerebral edema is consistent with several other observations:

1) The time course of VEGF in hypoxic rat and rabbit brain is similar to that encountered in the development of symptoms and signs of HACE in humans ascending too rapidly to high altitude.

2) Dexamethasone prevents or relieves AMS and HACE⁷ and also inhibits angiogenesis.⁸ Cultured bovine aortic and pulmonary artery endothelial cell monolayers subjected to hypoxia showed an increase in monolayer permeability which was prevented by addition of dexamethasone before or during hypoxia. Dexamethasone is commonly used to inhibit tumor growth by blocking angiogenesis. Its mechanism of action on cerebral edema has been unknown. It is not yet known whether the drug inhibits expression of VEGF or limits its action on basement membrane, but either action may explain its effectiveness in preventing and treating AMS and HACE.

However, there is little evidence of effective angiogenesis in brain during hypoxia. In rat brain after 3 weeks of moderate hypoxia (380 torr), LaManna et al found only increased capillary length, not number, and no budding capillaries.⁹

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CHAPTER 32

HIGH ALTITUDE DIURESIS: FACT OR FANCY

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Introduction

The renal response to hypoxia has intrigued physiologists and mountaineers since the earliest scientific studies of adaptation to high altitude. Despite much intensive field and laboratory investigation, the responses of the kidney and its role in acclimatization remain uncertain. Of considerable interest is the phenomenon of high altitude diuresis, which in combination with negative fluid balance is generally thought to be a feature of successful adaptation.²¹ The occurrence of high altitude diuresis and natriuresis, their physiological basis and role, if any, in the tolerance of high altitude will be the focus of this chapter.

The first report of diuresis with acute hypoxia was in 1910 by von Wendt⁵² who measured his own urinary output and electrolyte excretion while climbing in the Alps. Sundstrom⁴⁶ also demonstrated diuresis in some subjects with acute hypoxia in the Colorado Rockies. Further field and laboratory work in the 1930s showed that acute hypoxia in many mammals could be both diuretic and natriuretic, although conflicting findings, (ie antidiuresis) began to emerge. Credit for first use of the term high altitude diuresis (Höhendiurese) belongs to von Stämpfli and Eberle,⁵¹ who reported in studies at the Jungfraujoch in the Swiss Alps in 1944, that subjects above 3000 meters experienced a diuresis (Fig. 1). Interestingly, they also reported that subjects tolerating high altitude maintained a greater urine output while those who reported feeling ill thereafter became oliguric.

In the following decades there were many additional studies with measurements of salt and water regulating hormones, confirming diuresis with hypoxia but increasingly more reports of antidiuresis as well. Hackett and colleagues^{20,21} in their studies of trekkers and climbers in the Himalayan region in the 1970s convincingly advanced the concept that successful adaptation to high altitude was associated with greater ventilation, and fluid and weight losses, in part due to greater urinary output, recalled by subjects as greater urinary frequency. Acute mountain sickness (AMS) symptom scores were shown to be closely correlated with fluid balance as judged by weight loss or gain. This paradigm remains an attractive one, but the evidence supporting differences in ventilation, ventilatory control and fluid losses with acute hypoxia as critical determinants of AMS and other edemas of high altitude remains controversial.^{24,34}

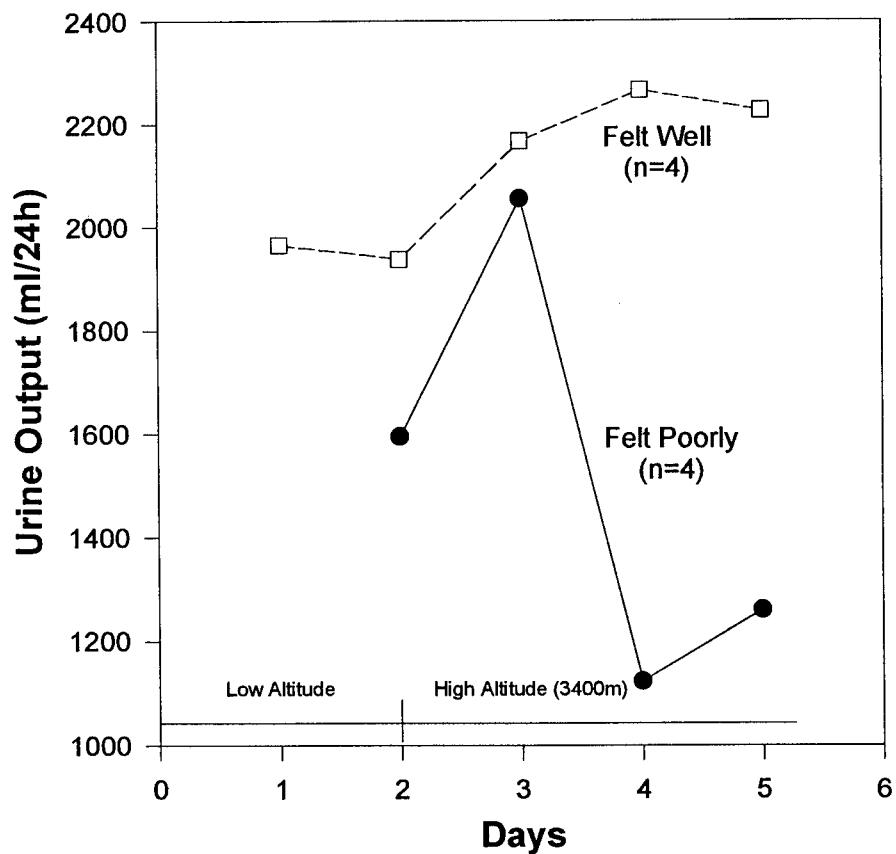


Figure 1 Höhendiurese (high altitude diuresis) in humans ascending to the Jungfraujoch in the Swiss Alps (von Stampfli and Eberle, 1944). Both groups had increased urinary output in the first twenty four hours, which was only sustained in those subjects remaining well.

Urinary output with acute hypoxia

As alluded to above, to date over 50 studies in humans and other mammals reveal a somewhat bewildering inconsistency of urine output in response to several minutes to many hours of acute hypoxia. The single greatest factor in this heterogeneity is the difference in the magnitude of inspired hypoxia. Figure 2 plots the human data from a variety of high altitude field studies, and laboratory studies of normobaric and hypobaric (chamber) hypoxic gas breathing, eliminating those studies in which there was explicit measurement or description of subjects becoming ill (reviewed in ref. 48). It is apparent from this analysis that there is a blurred but modest and biphasic dose response relationship. Above an F_1O_2 of 0.16 (or altitude equivalent) there is generally no effect, below which and down to an F_1O_2 of 0.12 most studies show a diuretic response. Between an F_1O_2 of 0.10 and 0.12, the magnitude of any diuretic effect diminishes and reverts to antidiuresis below an F_1O_2 of 0.10. Surprisingly there have been no controlled dose response studies in either humans or animals. The marked blurring of the dose response in this interstudy analysis of the human reports

(with both diuresis and antidiuresis occurring at equal F_1O_2), likely represents important uncontrolled factors such as differences in rate of 'ascent', duration of hypoxic exposure, body position, physical activity, preceding fluid and salt intake, bias in subject recruitment, and unreported AMS in some subjects. The last factor is quite important since it is clear that severe unpleasant stress and illness may themselves promote a state of avid salt and water retention.

When a hypoxic diuretic and natriuretic response occurs it can be detected within minutes and may last for many hours to several days. The composition of urine during the diuretic phase is generally one of mild variable hypotonicity and increased pH, with increased excretion of sodium, potassium, bicarbonate and chloride.^{17,47} The changes in acid-base composition are those expected with the renal response to acute hypocapnia, which accompanies hypoxemia in subjects permitted to increase their ventilation with hypoxia.¹⁷ When increased ventilation and respi-

Urinary Na⁺ Excretion with Hypoxia In Humans

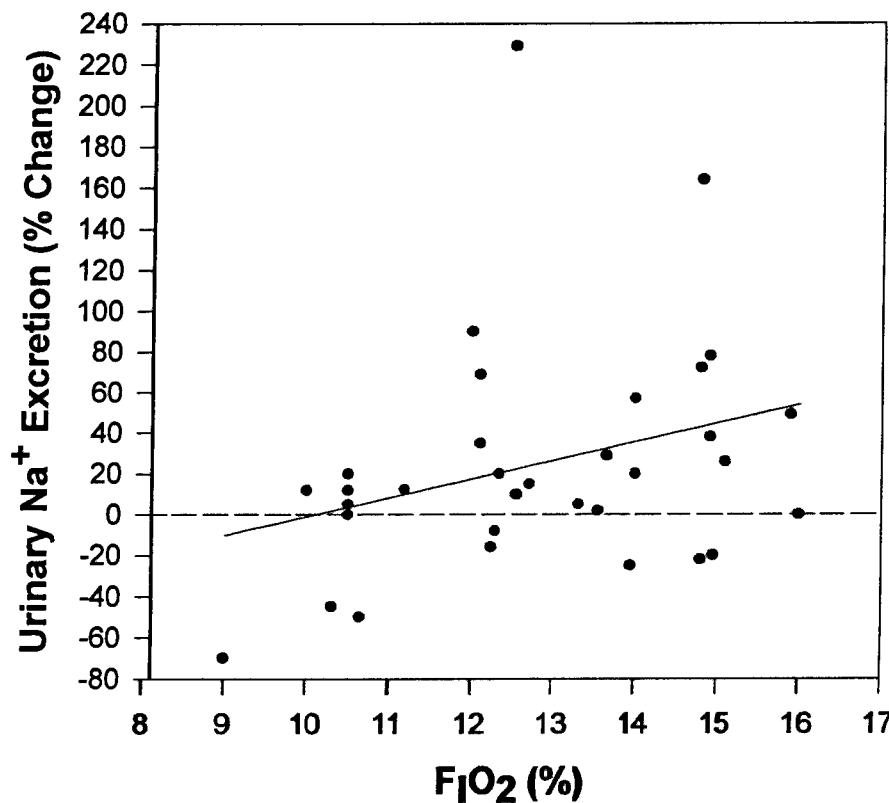


Figure 2 Compilation of 32 studies in humans with acute hypoxia showing percent change in urinary sodium excretion with various inspired oxygen concentrations (or altitude equivalents) measured over one hour to one day of acute hypoxia compared to normoxic baseline values.

ratory alkalosis are prevented in anesthetized animals, bicarbonate excretion is minimal and the losses are mainly sodium, chloride and water.²⁵

Although a detailed discussion of renal salt and water transport is beyond the scope of this review, it should be appreciated that acute hypoxia will alter a number of factors which regulate transport events in the kidney and cause or oppose diuresis under certain conditions. These include effects on systemic hemodynamics, global and intrarenal perfusion, hormones, acid-base status, and renal sympathetic innervation. These are discussed by Olsen in detail elsewhere in this volume.

Mechanisms of High Altitude Diuresis

Factors unrelated to hypoxia at high altitude can promote diuresis. These include hypobaria, however, Epstein and coworkers¹³ have demonstrated that normoxic hypobaria itself does not alter renal function or urinary output. Cold and hypothermia cause peripheral vasoconstriction and redistribution of blood volume into the central circulation, leading to a 'cold diuresis' well described in the hypothermia literature. However, the necessary fall in body temperature is likely not relevant to many climbers well dressed for alpine conditions. Normoxic isocapnic hyperpnea, or increased ventilation alone, can initiate a diuresis (see chapter by Olsen.) However, in the studies documenting this, ventilation at rest usually must exceed 20 l/min, values much in excess of the usual resting poikilocapnic hypoxic ventilatory response in resting conditions.³⁷ Normoxic respiratory alkalosis leads to a bicarbonaturia with an obligatory salt and water loss. Although any of these factors independent of hypoxia are capable of causing a diuresis, studies in anesthetized, temperature controlled and mechanically ventilated animals under normobaric conditions demonstrate that hypoxia alone is sufficient and critical for high altitude diuresis.²⁵

High altitude diuresis is likely not a result of isolated CNS hypoxia, since this evokes activation of the sympathetic nervous system leading to renal vasoconstriction, reduced GFR, antinatriuresis and antidiuresis.^{14,18} The only known innervation of the kidney is that of the sympathetic nerves.¹² Thus any generalized activation of the sympathetic nervous system is associated not only with renal vasoconstriction but catecholamine stimulated tubular reabsorption of sodium.¹² Isolated renal hypoxia is not a necessary factor. Although perfusions of the isolated kidney with hypoxic blood or saline cause renal vasodilation and diuresis and natriuresis,^{16,45} the levels of arterial desaturation necessary to do so (0-50%) would in the intact animal elicit intense sympathetic-mediated renal vasoconstriction and antidiuresis. Diuresis and natriuresis are not likely due to a generalized hypometabolic effect in the kidney, since natriuresis and diuresis *in vivo* occur without a fall in renal oxygen consumption and antinatriuresis only occurs when renal O₂ delivery and oxygen consumption drop.^{7,19} Although arterial hypertension does develop in some individuals and species with hypoxia and, itself, can cause a 'pressure' diuresis and natriuresis,⁴³ in many species, including man, diuresis occurs in the absence of arterial hypertension.⁴⁷ In contrast, however, antidiuresis always ensues in those subjects who become hypotensive with hypoxia, due to intense sympathetic nervous system activation.

The peripheral chemoreceptors are intimately involved in most responses of the body to hypoxia and renal function is no exception. The pioneering work of Honig and colleagues²⁵ established that high altitude diuresis, if and when it occurs, is a

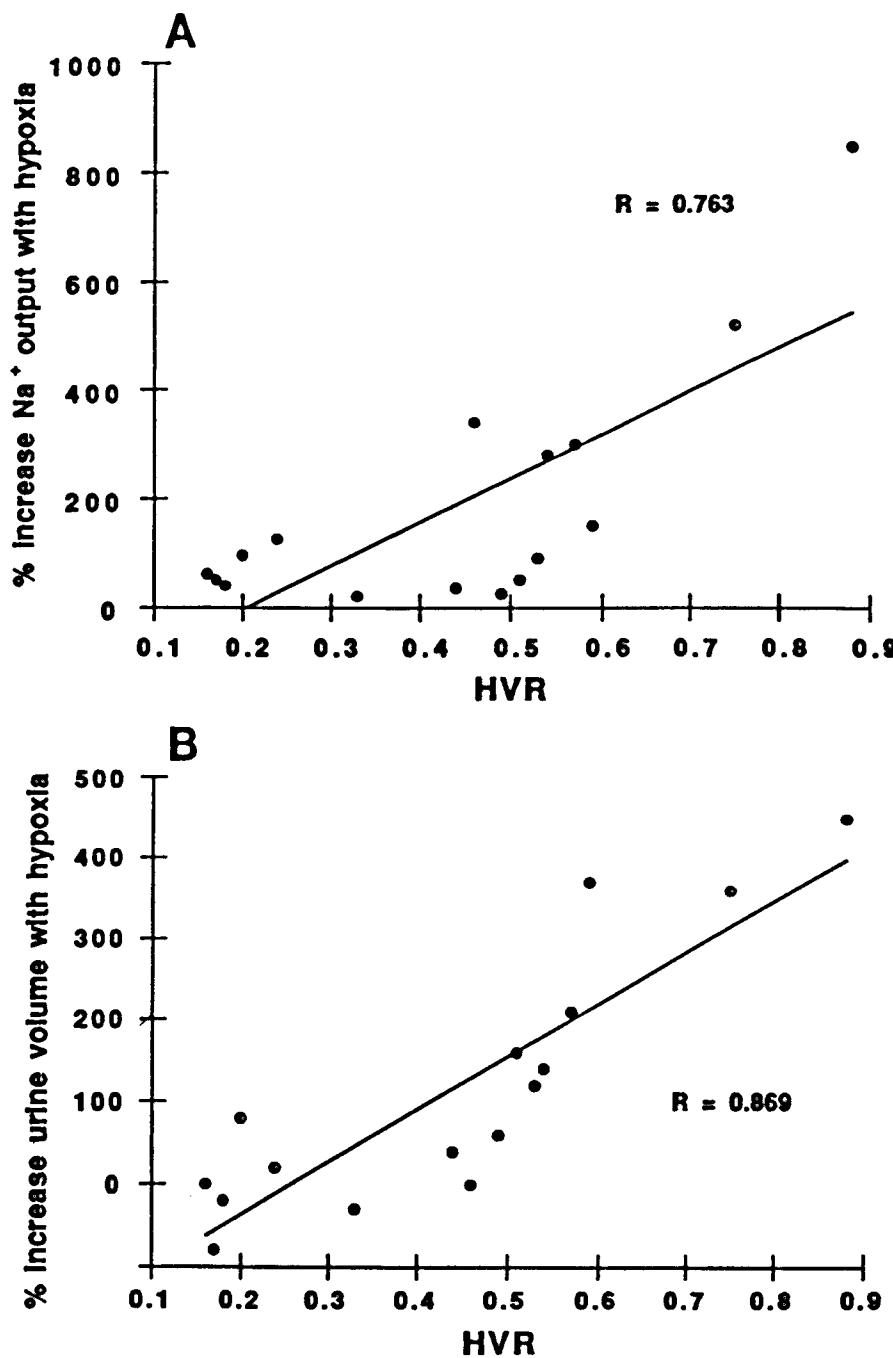


Figure 3 Correlation between isocapnic hypoxic ventilatory response (HVR) and increase in urinary sodium excretion (A) and urinary volume (B) with 6 hours of hypoxia ($O_2 = 14\%$) in 16 healthy human subjects. Correlation coefficients (R) are given.

result of peripheral chemoreceptor stimulation. Isolated hypoxic perfusion of the carotid body in animals ventilated with normoxic gas and fixed tidal volumes (to prevent hypocapnia and hyperpnea) leads to diuresis and natriuresis²⁵ which is abolished by carotid body denervation, and increased with renal denervation. Similar results have been demonstrated with almitrine, a peripheral chemoreceptor stimulant, in normoxic animals⁴ and humans.²⁷ Similar manipulations of the peripheral chemoreceptors is not possible in humans, but Swenson et al⁴⁷ showed that an individual's isocapnic hypoxic ventilatory response (a measure of peripheral chemosensitivity) was strongly correlated to the magnitude of his or her diuretic and natriuretic response to six hours of breathing 14% oxygen (Fig. 3).

Although it is clear that the peripheral chemoreceptors are involved in high altitude diuresis, the effector mechanism(s) remain unknown. A mediation via the renal nerves is unlikely since denervation of the kidneys leads to greater diuresis.²⁵ It is known that peripheral chemoreceptor stimulation increases renal sympathetic nerve activity as part of the general activation of the sympathetic nervous system with hypoxia.³¹ With renal denervation the natriuretic stimulus to the kidney is unopposed and augmented, thus a humoral mediator has been sought. Numerous studies of known salt and water regulating hormones such as aldosterone, atrial natriuretic peptide, angiotensin II, vasopressin, cortisol, and circulating catecholamines have been unrevealing. Although some studies show appropriate changes in several of these hormones, none are consistently associated with the response.⁴⁸

However, the above list is incomplete as more and more substances with natriuretic effect are discovered. Of those known to be elevated in the circulation and/or urine with hypoxia, brain natriuretic peptide (BNP),^{23,49} digitalis-like immunoreactive substance (DLIS),¹⁰ endothelin,⁴⁰ adrenomedullin,²⁶ and urodilatin²⁸ are possible candidates. BNP, DLIS and adrenomedullin are interesting possibilities since they are found in the hypothalamus and adrenal gland, sites in addition to the brain stem to which the peripheral chemoreceptors project. Circulating endothelin concentrations rise two to three fold with acute hypoxia.⁴² At these concentrations, endothelin may be natriuretic by its direct suppression of tubular sodium reabsorption⁵⁴ or by local stimulation of intrarenal nitric oxide release.⁴² Endothelin may also be natriuretic by its stimulation of renal medullary blood flow,⁴⁴ which leads to an increase in renal interstitial pressure and inhibition of tubular sodium reabsorption.⁴³ Urodilatin, a peptide related to ANP and BNP, is only found in the kidney and appears in the urine with hypoxia.^{6,28} However, unlike BNP and DLIS, neural projections (apart from the renal sympathetic efferents) from the peripheral chemoreceptors to the site of endothelin and urodilatin formation have not been established. Other interesting potent natriuretic hormones worthy of investigation include medullipin,³⁸ uroguanylin,¹⁵ and neuropeptide Y,¹⁵ but it is not known whether hypoxia affects their production.

As Figure 2 shows, antidiuresis and antinatriuresis can occur at almost any level of hypoxia including those that in other studies lead to marked diuresis and natriuresis. Clearly at some point the absolute severity of hypoxia evokes sufficient antinatriuretic and antidiuretic stimuli (hypotension and intense sympathetic nervous system activation). This likely varies among individuals just as do other hypoxic responses such as pulmonary vasoconstriction,³⁹ ventilation,^{24,34,47} and urinary output.⁴⁷ These studies show large differences in inter-individual responses, which may vary as much as tenfold.

Another important factor that may explain such antipodal responses to equivalent degrees of hypoxia between studies is the level of physical activity. All animal work demonstrating hypoxic diuresis involved either anesthesia or measurements in resting or restrained conscious subjects. Similarly the human data are heavily weighted by studies either involving normobaric hypoxia in the laboratory, passive ascents to high altitude, or simulations in hypobaric chambers in which no significant physical exertion was involved. One important effect of exercise is to cause greater arterial desaturation than that at rest, therefore increasing the hypoxic stress at any equivalent altitude. Exercise at sea level elicits antidiuretic and antinatriuretic effects, including increased renal sympathetic nerve activity³² and elevations in salt and water retaining hormones such as aldosterone, circulating catecholamines and those of the renin-angiotensin system.³⁶ The result is marked reductions in GFR, renal blood flow, urinary volume and sodium excretion.⁴¹ This antinatriuretic and antidiuretic state may persist for several hours following exercise.⁸ The pattern with hypoxic exercise is similar although a larger rise in atrial natriuretic factor and lesser rise in aldosterone is often observed.^{2,29,41}

The physical exertion of climbing, therefore, may oppose peripheral chemoreceptor-mediated diuresis and natriuresis and dominant fluid balance and urinary responses at high altitude. This has been very clearly demonstrated in the studies of Milledge and co-workers who showed that daylong moderate 'hill walking' at intermediate altitudes is associated with activation of the renin-angiotensin-aldosterone system, reduced urinary water and sodium excretion, and increased fluid retention.^{33,35} In contrast, Loepky et al³⁰ showed that enforced minimization of activity (bed rest) caused even greater diuresis and natriuresis than normal indoor sedentary activity at high altitude. Thus in studies of humans climbing to high altitude, only those with slower ascent rates and long periods of rest,^{1,50} several immediate preceding days of altitude exposure in passive transit to the trailhead³⁴ or data beyond 4-5 days of continuous climbing^{11,22} show negative fluid balance and increased urinary sodium and water losses. These findings suggest that with acclimatization, the antinatriuretic and antidiuretic effects of exercise abate and a negative fluid balance develops in those who do not become ill.

Figure 4 presents a scheme of the relevant factors involved in the renal response to high altitude, highlighting both those elements that promote natriuresis and diuresis and those that oppose them and lead to antinatriuresis and antidiuresis. Figure 5 shows that the ultimate outcome in any one individual will be determined by the magnitude and balance of these competing influences. Given the complex and as yet incompletely understood multisystem control of renal function in hypoxia it is not surprising that it has been difficult to reconcile much of the literature and predict with certainty how individuals will respond at high altitude.

Relevance of High Altitude Diuresis

Several possible advantages to an early diuretic response to high altitude can be envisaged. The first is an increase in the hematocrit and blood O₂ carrying capacity as the extracellular volume is reduced. This increase in hematocrit can occur early (minutes to hours) well before any erythropoietin mediated increase in erythropoiesis brings new red cells into the circulation (several days). In addition, a slightly reduced intravascular volume may help lessen the total pressure-volume load on the pulmonary circulation occurring with the increase in cardiac output and

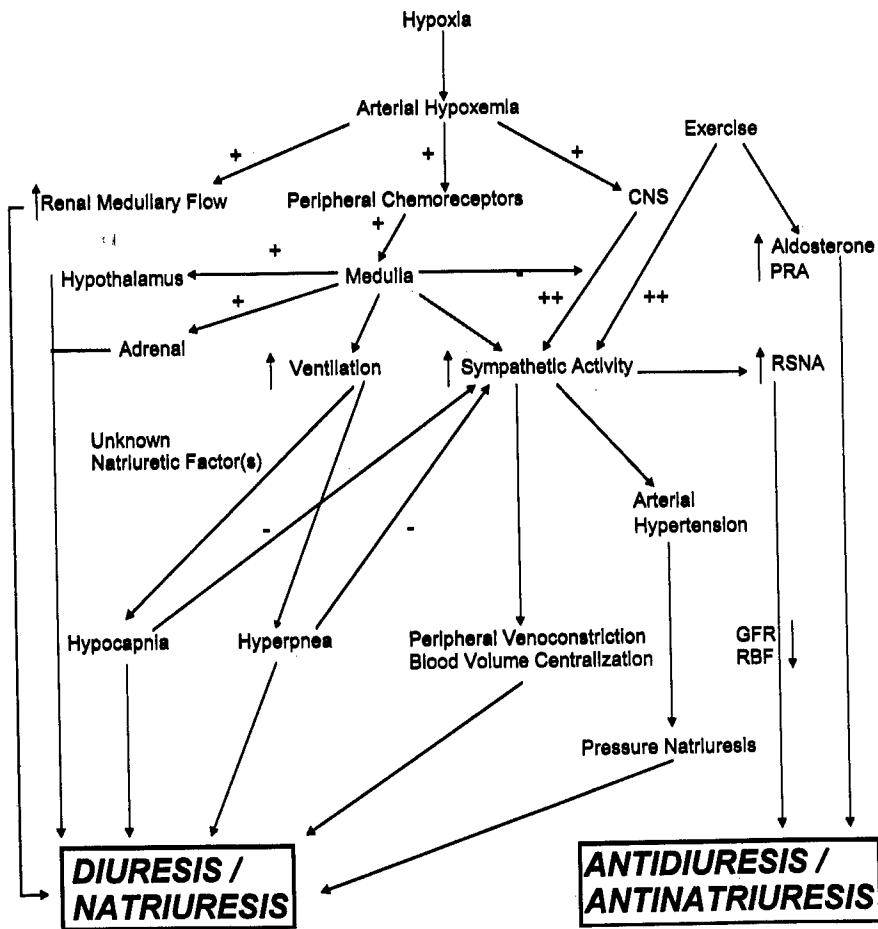


Figure 4 Multiple pathways by which hypoxia can affect renal function leading to both diuresis and natriuresis in some circumstances or to antidiuresis and antinatriuresis in others. (+) symbol represents stimulatory and (-) symbol represents inhibitory influences. PRA = renin-angiotensin-aldosterone system, RSNA = renal sympathetic nerve activity, GFR = glomerular filtration rate, RBF = renal blood flow.

increased pulmonary vascular resistance, from both sympathetic and hypoxic vasoconstriction. Lastly, a preemptive loss of salt and water would help to minimize the tendency toward lung and brain edema formation.

However compellingly attractive these advantages may appear, it is not established that any of these responses limit AMS or the edemas of high altitude. There are no adequate studies which show poorly acclimatizing subjects have preceding antidiuresis before the appearance of AMS symptoms. The more usual finding is that antidiuresis and positive fluid balance follow the development of AMS. If a peripheral chemoreceptor mediated high altitude diuresis is adaptive, then a measure of peripheral chemosensitivity such as the HVR, should, in addition to its prediction of ventilatory response at high altitude, predict the likelihood of negative fluid balance and AMS. Yet, a number of studies have not found support for this concept,

Renal response to high altitude Diuresis vs. anti-diuresis

Diuresis

high peripheral chemosensitivity
hypocapnia
volume replete state
rest or mild activity
modest sympathetic response

Anti-diuresis

low peripheral chemosensitivity
little or no hypocapnia
low Na^+ intake/volume depletion
exercise
intense sympathetic response



Figure 5 Simplified scheme highlighting factors promoting a high altitude diuretic response vs. those leading to an antidiuretic response.

at least as HVR is conventionally measured at sea level.^{24,34} Bärtsch et al.³ directly tested whether HVR could predict urinary responses in climbers as shown in non-climbing subjects by Swenson et al.⁴⁷ In contrast, they found that low altitude testing of HVR did not predict sodium and water excretion in climbers ascending to 4459 m in the Alps. They also found that differences in fluid balance between the well acclimatizing subjects and those with AMS (ie greater sodium and water excretion) only became apparent after the subjects with AMS were ill, at which point their urinary sodium and water excretion fell. Biollaz et al. (1996, unpublished observations) found that with rapid passive ascent (airlift) to 4459 m, subjects who developed AMS (within ten hours of arrival) did not differ in renal sodium and water excretion in the first 2 days at altitude from those who remained well. The design of the study included a five day stabilization of sodium and water intake which was continued at high altitude, no physical activity and long periods of recumbency. Surprisingly the subjects developing AMS had an equivalent diuresis and natriuresis as the subjects remaining healthy in the first 48 hours, suggesting that the occurrence of AMS does not require a period of fluid retention and that it can occur despite a negative fluid balance.

Conclusions

High altitude diuresis or Höhendiurese is an interesting physiological response, the facts of which are that it occurs in humans and animals under hypoxic conditions generally associated with F_1O_2 not lower than 0.12 and in the absence of any significant physical exertion. It is largely mediated by the peripheral chemoreceptors, although the translation of their afferent signal is unknown. Renal denervation studies rule out a direct neural communication to the kidneys, but as yet no circulating hormonal mediator has been unequivocably found. At this point, the response is either driven by release of an unknown natriuretic hormone, possibly from the hypothalamus or adrenal gland, both of which receive projections from the peripheral

chemoreceptors. On the other hand, given the complexities of the intrarenal circulation, it can not be ruled out that subtle changes in the distribution of blood flow with redistribution to the medullary circulation may not be responsible. The difficult question remains as to how this occurs in the face of increased renal sympathetic nerve activity. High altitude diuresis can be overridden by intense activation of the sympathetic nervous system such as occurs with exercise and/or severe hypoxic stress. Despite physiological plausibility, careful examination of the older literature and now more recent studies suggest the fancy that a lack of high altitude diuresis precedes the development of AMS and is a critical determinant in the pathophysiology of AMS is not true.

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CHAPTER 33

VENTILATION, HYPOCAPNIA AND HYPOXIA: EFFECTS ON RENAL FUNCTION

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Introduction

One of the main functions of the kidneys is to maintain body fluid homeostasis by regulation of water and electrolyte balances, body fluid osmolality, and acid-base balance. In addition, renal excretion of sodium and water is the primary means for regulation of arterial blood pressure. In most species, hypoxemia causes pronounced changes in respiratory, cardiovascular and endocrine function and in body fluid composition. Hypoxemia also affects renal function either because of specific, intrarenal effects or as part of a generalised cardiovascular response or both. This chapter focuses on the functional effects of acute, short-term hypoxic exposure on renal hemodynamics, glomerular filtration rate (GFR) and tubular function.

Overall effects of short-term hypoxemia on renal function in spontaneously breathing mammals and humans

When acutely exposed to hypoxic gas or to hypobaric hypoxia, conscious animals and humans allowed to breathe spontaneously within the first minutes develop hyperventilation, which after a few more minutes results in hypocapnia and respiratory alkalosis.^{64,76,82} Cardiac output increases mainly by an increase in heart rate.^{45,48,73,78,96,97} In rats, acute hypoxemia (6-15% O₂ for 3 to 30 min.) is associated with a pronounced fall in mean arterial pressure.^{7,19,52,63,64} In the presence of this depressor effect of hypoxemia, some studies revealed an increase in renal perfusion^{52,64} whereas others reported a decrease in renal blood flow and an increase in renal vascular resistance.⁷ However, both groups reported a concomitant decrease in GFR and in renal excretion rates of sodium and water.^{7,52,64} In contrast, Colice et al found that hypoxic breathing in conscious rats induced a natriuretic and diuretic response.¹⁹

In conscious, spontaneously breathing dogs, acute hypoxemia induces an increase in arterial pressure^{45,48,97} that instantly and until a new steady state has been reached would be expected to evoke the phenomena of pressure diuresis and pressure natriuresis (Fig.1). In line with this, Walker showed that acute, hypocapnic hypoxemia caused a pronounced increase in renal sodium excretion rate and urinary output, but also that this response was associated with an increase in renal blood flow and GFR.⁹⁷ In the presence of intact renal autoregulation, such a response is not

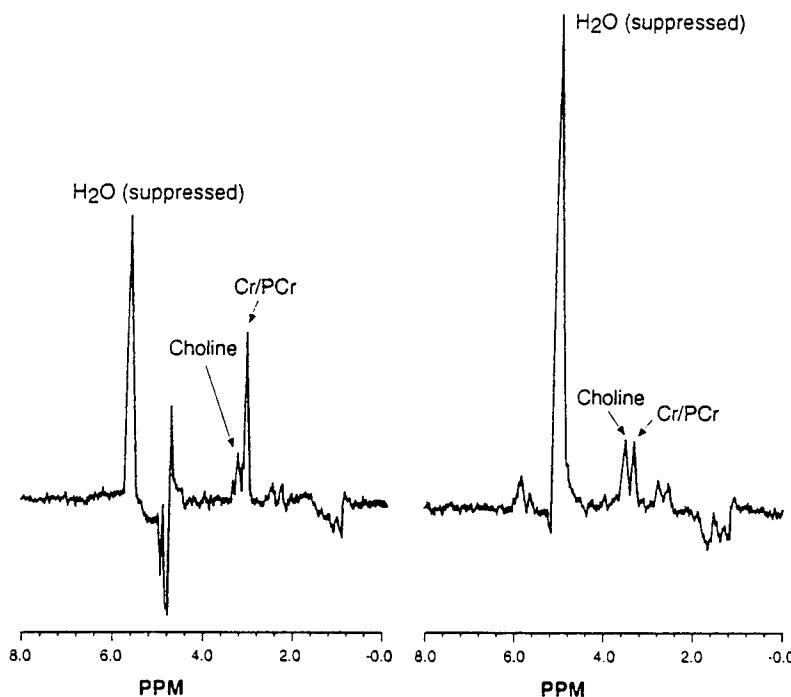


Figure 1 Autoregulation of renal blood flow (RBF, left curve) and glomerular filtration rate (GFR, middle curve) but not of urine flow (right curve) during changes in arterial blood pressure. Note that glomerular filtration ceases when hydrostatic pressure falls below the sum of colloid osmotic pressure and tubular pressure.

normally observed during increases in arterial pressure (Fig. 1). The exact tubular mechanisms responsible for the natriuresis associated with acute increases in arterial pressure remain unclear, but may be related to a decrease in tubular reabsorption of sodium and water secondary to an increase in the renal interstitial fluid hydrostatic pressure.^{35,37,67} Others also reported that acute hypoxemia in conscious dogs increased renal blood flow.^{45,48,56} However, GFR remained unchanged.^{31,56}

In humans acutely exposed to hypoxia, arterial pressure normally does not change^{8,15,18,73,95} or decreases slightly.³⁹ Table 1 shows results obtained in our laboratory from normal subjects exposed to hypoxic breathing with 10% O_2 for two hours (Christensen et al, unpublished results). Only few studies have investigated the renal response to acute hypoxemia in humans. Repeated exposures to a simulated altitude of 18,000 feet for two hours three times weekly for eight weeks caused a temporary natriuresis and diuresis, that after each exposure "...was compensatorily reduced so that total excretion during the 24 hours was not altered."¹⁴ Hypoxic gas breathing in healthy subjects induced a slight increase in renal blood flow, which was accompanied by a natriuretic and diuretic response.⁸ Others found that the renal blood flow either increased⁹ or remained unchanged.^{1,15} A constant finding has been that hypoxemia in humans only induces small, if any changes in GFR.^{1,8,9,15,92} In accordance with this, our studies revealed that hypoxia-induced natriuresis was associated with an increase in the effective renal plasma flow without changes in GFR

Table 1

Arterial blood gases, ventilation, and systemic hemodynamics in normal subjects exposed to two consecutive 1-hour periods of hypoxic gas breathing with 10% O₂ (hypoxia 1 and 2).

	Baseline	Hypoxia 1	Hypoxia 2	Recovery
PaO ₂ (mmHg)	104±3	40±2***	37±4***	102±2
PaCO ₂ (mmHg)	41±1	35±2***	33±2**	38±1**
VE (l/min)	8.2±0.9		14.7±1.2***	10.6±1.5*
Heart rate (bpm)	50±2	63±3 *	61±4 *	49±2
MABP (mmHg)	99±5	94±3	93±3	94±4
Cardiac output (l/min)	4.2±0.5		6.2±0.6 **	4.2±0.5
TPR (mmHg min l ⁻¹)	28.6±4.7		17.3±1.6 *	27.6±3.8

Values are means with V_{SEM}. N=8. V_E, respiratory minute volume; MABP, mean arterial blood pressure; TPR, total peripheral resistance. *) P<0.05, **) P<0.01, ***) P<0.001 compared with baseline. (Christensen et al, unpublished results).

(Fig. 2). Importantly, it has been demonstrated that the response to hypoxemia may vary considerably between and within the subjects,^{15,39} and that oxygen fractions around 10% in some subjects may activate stimuli that cause hypotension, nausea and increased secretion of antidiuretic hormone, which subsequently results in an antidiuretic response.³⁹ Similar observations, referred to as the autonomic components of an alerting/defence response, have also been made in the rat;⁶⁴ obviously, the occurrence of such responses to acute hypoxemia may confound the results obtained in conscious animals and humans.

At least six distinct factors may interact to produce the integrated renal response to acute hypoxemia in spontaneously breathing animals and humans:

1. Changes in renal perfusion pressure
2. Direct intrarenal effects of hypoxia on tubular function and renal autoregulatory mechanisms
3. Local effects of hypoxia on renal vessels
4. Reflex effects of hypoxemia including chemoreceptor reflexes, the lung inflation reflex and renal sympathetic nerve activity
5. Effects of the accompanying decrease in arterial CO₂ tension
6. Humoral changes

Changes in renal perfusion pressure

When present, acute hypoxia-induced changes in arterial blood pressure and thus in renal perfusion pressure should be expected to be met instantly by an appropriate response of the renal-body fluid system, so that even a small increase in arterial pressure is compensated by a decrease in the renal tubular reabsorption of sodium and water, and *vice versa* (Fig. 1). The antidiuretic and antinatriuretic response to acute hypoxemia normally observed in the rat can largely be explained as secondary to the fall in renal perfusion pressure. In support of this, the antidiuresis and antinatriuresis could be totally abolished in hypoxic rats when the renal perfusion pressure was prevented from falling by clamping the distal aorta.⁶⁴ This maneuver had the same effect in innervated and denervated kidneys and apparently did not interfere

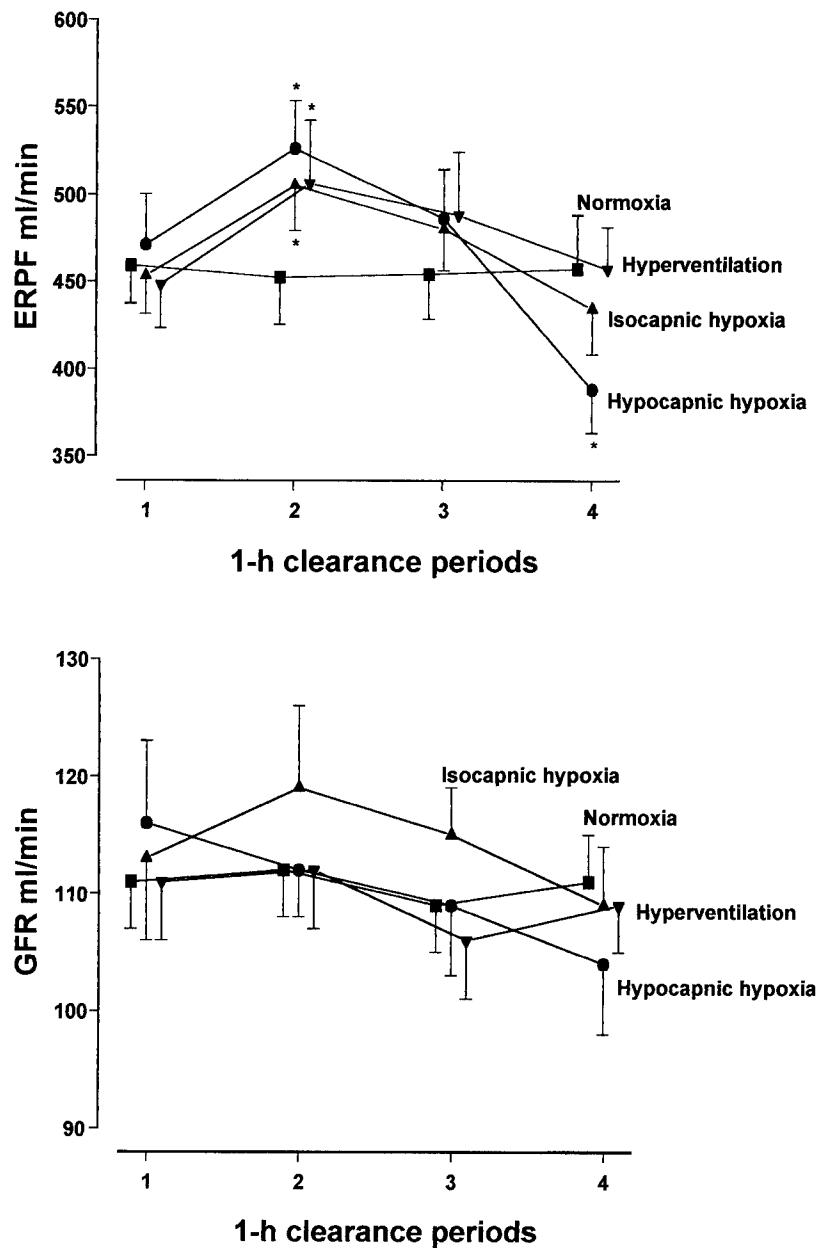


Figure 2 Effects of hypoxic and normoxic hyperventilation on effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) in normal subjects. In each subject, the different gas mixtures were administered during two consecutive 1-h periods on separate study days. Normoxic hypoxia was achieved by addition of CO_2 to the hypoxic gas. Hyperventilation was adjusted to produce a similar decrease in P_aCO_2 as during hypoxic hypoxia. Period 1, baseline; periods 2 and 3, experimental periods; period 4, recovery. Values are means with SEM, $N=8$. * $P < 0.05$ compared with normoxemia (normal breathing of room air through the same tight-fitting face mask as used on the other study days). (Christensen et al, unpublished results).

with a local vasodilating effect of hypoxemia on renal vessels which persisted with and without distal aortic occlusion.⁶⁴ The same mechanism, however now operating in the opposite direction, is likely to be of predominant importance for the diuresis and natriuresis observed in the presence of an elevated arterial pressure in hypoxicemic dogs. When the pressor response to hypoxemia is blunted by pretreatment with the β -adrenergic receptor antagonist propranolol also urinary output is diminished.⁹⁷

Hypoxic diuresis and natriuresis, however, may occur in the presence of an unchanged or decreased arterial pressure.^{8,15,19,39,95} Although this emphasizes that mechanisms other than a pressure-related response are activated, there is no evidence to indicate that the renal response to changes in renal perfusion pressure when present can be overpowered by such mechanisms. On the contrary, the available evidence indicates that when a hypoxia-induced change in renal perfusion pressure occurs, it will predominate in determining the overall renal excretory response.^{64,97}

Direct intrarenal effects of hypoxia on tubular function and renal autoregulatory mechanisms

From the renal cortex to the medulla there is a steep gradient of oxygen with very low tensions of oxygen at the tips of the renal papillae.¹¹⁻¹³ This corticomedullary gradient of oxygen results from the special arrangement of the peritubular capillaries, the *vasa recta*, that as part of a countercurrent system is lying side by side with the loops of Henle of the juxamedullary nephrons. The system is essential for the urinary concentration mechanism, but also forms the basis for a countercurrent diffusion of oxygen between the descending and ascending parts of the *vasa recta*. This together with a high metabolic rate linked to the active transport mechanisms in the medullary thick ascending limb is supposed to render this part of the kidney especially susceptible to hypoxic insults.^{11-13,27} About 75% of the renal oxygen consumption is related to the tubular reabsorption of sodium, and evidence exists to suggest that the kidneys, by an intrinsic protective mechanism, react to an oxygen deficit by reducing tubular reabsorption.^{13,28,94} This tends to restore the balance between the availability and consumption of oxygen in the medulla and in turn also results in a downregulation of GFR: the increased delivery of tubular fluid and solutes to the *macula densa* activates the tubuloglomerular feedback mechanism, and the increase in the tubular intraluminal pressure reduces the hydrostatic filtration pressure gradient across the glomerular capillaries.^{41,94} This secondary response further protects the kidneys against cellular hypoxia by reducing the metabolic workload imposed by the filtered load and also effectively serves to prevent the reabsorptive failure from leading to an excessive loss of fluid and solutes.⁹⁴

In the isolated, perfused rat kidney, the intrarenal effects of hypoxemia can be studied in the absence of concurrent changes in systemic hemodynamics, renal nerve activity, and humoral factors. Consistent with the concept of an intrinsic protective mechanism, hypoxic perfusion caused marked decreases in fractional sodium reabsorption and GFR.^{33,36,61} Urine flow rate initially increased and then decreased in parallel with GFR to very low values.³³ Indirect assessments of segmental tubular function indicated a depressing effect of hypoxia on both proximal and distal tubular transport mechanisms.³⁶ In dogs, *in vivo* perfusion of the kidney with venous blood from the right ventricle increased excretion rates of sodium and water and, on average, slightly decreased GFR.⁸⁷ Adenosine, formed by ATP hydrolysis, may play a major role in the regulation of energy-dependent tubular reabsorption processes. In

outer medullary and thick ascending limb suspensions from rats, graded hypoxia increased adenosine release,⁶ and in the isolated, perfused medullary thick ascending limbs of the rat, adenosine reduced absorption of chloride.⁵ In addition, increased tubular adenosine production may enhance tubuloglomerular feedback.⁸⁶ Another substance likely to be involved is endothelin that has been shown to accumulate in renal tubular epithelial cells when anesthetized dogs were exposed to hypoxic gas.⁶⁵ This was associated with a diuretic and natriuretic response without changes in renal hemodynamics.⁶⁵ Both tissue hypoxia and hypoxemia stimulate the expression of the endothelin-1 gene in the rat kidney.⁷⁷ Although endothelin is a potent vasoconstrictor in systemic and renal circulations, low exogenous doses of endothelin can increase urinary output and sodium excretion.^{43,85} Accordingly, endothelin has been shown to inhibit Na^+-K^+ -ATPase activity in proximal tubules and to decrease oxygen consumption in inner medullary collecting ducts.¹⁰⁰

Thus, the available evidence indicates that the kidneys during incipient hypoxia are capable of reducing energy-dependent solute transport in the tubules by direct mechanisms that are likely to involve adenosine and endothelin and that in turn may decrease the filtered load by activation of the tubuloglomerular feedback mechanism. It remains unknown, however, whether hypoxia *per se* interacts with the efficacy of renal autoregulatory mechanisms and to what extent the intrinsic protective mechanisms contribute to the observed changes in overall renal function following systemic hypoxemia. Like other organ systems, the kidneys can take advantage of alternative, anaerobic metabolic pathways for ATP formation that during severe hypoxia can ameliorate the decrease in tubular and glomerular function.³⁶ During mild to moderate hypoxia mobilization of these pathways may be sufficient to fully maintain an adequate ATP production.

Local effects of hypoxia on renal vessels

Several studies in unanesthetized, spontaneously breathing rats,^{52,63,64} dogs^{45,48,56,97} and humans^{8,9} demonstrated that hypoxemia may increase renal blood flow and/or decrease renal vascular resistance. This was also found in our study (Fig. 2). Interestingly, hypoxic breathing in normal subjects has been found to decrease splanchnic vascular resistance, which normally in response to physical stress changes in the same direction as in the kidneys.⁸³ Although neural and humoral factors are likely to be involved (see below), locally induced vasodilating effects of hypoxia on the renal vasculature may as well contribute. It is well documented that hypoxemia by local mechanisms can induce vasodilation in cerebral, coronary, and skeletal muscle circulations and vasoconstriction in pulmonary vessels,⁸² but the renal (and splanchnic) response is far less understood. Some studies in dogs using local renal hypoxic perfusion indicated the presence of a vasodilating effect^{87,91} whereas others did not.^{24,38} Nor did hypoxemia change renal blood flow in adrenalectomized rabbits pretreated with guanethidine and atropine.¹⁶

In isolated renal, femoral, and saphenous arteries of the rabbit, hypoxia reduced both potassium and norepinephrine-evoked contractions, but with norepinephrine the renal artery contraction was affected to a lesser degree than in femoral and saphenous arteries.⁵⁰ In the *in vitro* perfused hydronephrotic rat kidney, graded reductions of PO_2 within the range of 60 to 20 mmHg progressively inhibited myogenic vasoconstriction of the afferent arteriole by activation of ATP-sensitive potassium channels.⁴⁹ In contrast, hypoxia in isolated small arteries of the dog kidney enhanced nor-

epinephrine-induced contractions.²⁹ This response was prevented by endothelial damage suggesting that hypoxia in renal vessels of the dog causes release of an endothelial-derived vasoconstricting mediator.²⁹ In anesthetized dogs, inhibition of endogenous endothelium-derived relaxing factor had no influence on the renal vasoconstricting response to hypoxemia.⁷²

Possible candidates for a role as renal vasoconstrictors are endothelin and adenosine. Circulating levels of endothelin, known as a powerful vasoconstrictor in renal vasculature,⁴³ are elevated in hypoxic conditions.⁷² Hypoxia also in most tissues causes release of adenosine,^{57,58,71,74,98} which is now recognized as an important mediator of hypoxia-induced vasodilation in the brain, heart, splanchnic, and skeletal muscle.^{55,57,58,62} However, in the renal circulation adenosine may elicit vasoconstriction by action mediated via A₁ receptors in afferent arterioles.⁷¹ On the other hand, Neylon and Marshall demonstrated that adenosine plays a role in the hypoxia-induced renal vasodilation observed in the rat, since adenosine receptor antagonists reduced the increase in renal vascular conductance.⁶³ However, the vasodilating action of adenosine seemed to be more pronounced in skeletal muscle than in the kidneys, and, furthermore, the results obtained during severe hypoxemia (F_iO₂ ~ 6%) suggested the influence of a renal vasoconstricting factor not observed during less severe hypoxemia.⁶³ This study also demonstrated that adenosine-induced vasodilation in muscles is responsible for a major part of the hypoxia-induced decrease in arterial blood pressure.⁶³ Renal vascular responses are likely to be modulated by changes in renal prostanoids, since *in vitro* hypoxic perfusion of the rat kidney increases the ratio of vasodilator to vasoconstrictor prostanoids by inhibition of TxB₂ release.⁶¹

Taken together, local renal vascular effects of hypoxia seem to differ between the rat and the dog and the issue is further complicated by the differential effects of adenosine on renal vessels as compared with other organ circulations. In the rat, the net effect of mild to moderate hypoxemia may result in vasodilation but to a lesser degree than in muscle, splanchnic, heart, and brain circulations. With severe hypoxia, the vasodilating effect may be opposed by renal vasoconstricting effects of adenosine. Whether or not local effects of hypoxemia contribute significantly to the renal vasodilation observed in humans^{8,9} (Fig. 2) remains unsettled.

Reflex effects of hypoxemia including chemoreceptor reflexes, the lung inflation reflex and renal sympathetic nerve activity

Graded levels of acute hypoxemia progressively increase renal sympathetic nerve activity (RSNA).^{32,88,89} The effect of hypoxemia on RSNA, however, is smaller than the effects on cardiac and pulmonary sympathetic nerve activity.^{32,89} Denervation of the carotid and aortic bodies abolishes the hypoxic increase in RSNA,^{32,89} indicating that the response is primarily mediated by peripheral rather than by central chemoreceptors. In contrast, the increase in cardiac and pulmonary nerve activity is largely caused by activation of central mechanisms.^{32,89} Also the marked increase in RSNA evoked by elevated arterial CO₂ levels is mostly mediated by central chemoreceptors.³² The role of peripheral chemoreceptors for cardiovascular regulation has recently been reviewed.⁵¹

Unopposed, a hypoxia-induced increase in RSNA supposedly would cause constriction of renal arterioles and an increase in renal tubular sodium reabsorption.^{25,60} As emphasized by Rowell,⁸² the overall cardiovascular response to chemoreceptor

activation during asphyxia and apnea resulting from diving or submersion differs substantially from the response to hypoxemia in animals and humans able to react with an increase in ventilation. In diving animals, the predominant pattern elicited by activation of arterial chemoreflexes consists of pronounced decreases in organ blood flow including the kidneys.^{82,99} However, in hypoxic animals allowed to breathe spontaneously, activation of pulmonary stretch receptors secondary to hyper-ventilation may completely inhibit the excitatory effect of chemoreflexes on the sympathetic discharge from the vasomotor center, thereby inhibiting the vasoconstrictor response to chemoreceptor stimulation.⁸² In conscious dogs, elimination of afferent pulmonary nerve activity by cervical vagal blockade increased renal vascular resistance during hypoxic breathing.⁴⁵ In anesthetized rabbits, hypoxia-induced renal vasoconstriction could be reversed to vasodilation by artificial hyperventilation.²² Also the vasoconstrictor response to chemical stimulation of peripheral chemoreceptors can be modulated by changes in ventilation.⁸⁴ It is well known that stimulation of mechanosensitive, intrathoracic receptors inhibits RSNA.⁶⁰ That this reflex may influence renal hemodynamics in normoxic conditions has been demonstrated in anesthetized dogs where normoxic hyperventilation decreased renal vascular resistance.⁴⁴ In humans, voluntary hyperventilation increases renal sodium excretion^{23,34} even if hypocapnia is prevented by CO₂ supplement.²³ In line with this, results from our laboratory shows that normoxic hyperventilation in normal subjects increases the effective renal plasma flow (Fig. 2) and sodium excretion.

In summary, stimulation of pulmonary stretch receptors by increased ventilation inhibits sympathetic vasomotor outflow and reduces RSNA. Activation of this reflex has been shown to attenuate the renal vasoconstrictor response to hypoxic chemoreceptor stimulation and may explain, in part, why hypoxemia in spontaneously breathing animals and humans does not result in marked regional vasoconstriction like that elicited by asphyxia and apnea.⁸² Possibly, activation of atrial stretch receptors, which also are inhibitory, may contribute secondary to acute pulmonary vasoconstriction and/or an increase in intrathoracic blood volume due to the increased ventilation. The significance of these reflexes, however, may vary within species, depending on the magnitude of the ventilatory response to hypoxemia and to the sensitivity of the Hering-Breuer reflex in the species.⁶⁶ In the rabbit, where both the hypoxic ventilatory response and the gain of the Hering-Breuer reflex seem to be smaller than in the dog, selective lung denervation only caused a minor increase of the RSNA response to hypoxemia.⁶⁶ In contrast to what has been observed in humans, normocapnic hyperventilation in normoxic rats did not increase sodium excretion.⁵⁹ Direct measurements of muscle sympathetic nerve activity in humans suggest that activation of pulmonary mechanoreceptors strongly reduces sympathetic outflow during hypoxic breathing.⁹⁰

Effects of the accompanying decrease in arterial CO₂ tension

The hypoxic ventilatory response inevitably results in hypocapnia that by itself may modulate the renal response to hypoxemia. In contrast to hypercapnia, which causes a marked increase in RSNA by stimulation of central chemoreceptors, hypocapnia has only minor effects on RSNA in normoxic conditions.³² In several studies, Rose et al clearly demonstrated that combined hypoxemia and hypercapnia in conscious dogs produces a pronounced decrease in renal blood flow.⁷⁸⁻⁸¹ This was also found by Koehler et al.⁴⁵ However, renal blood flow increased during hypocap-

nic hypoxemia but remained unchanged when the hypocapnia was prevented by addition of CO₂ to the hypoxic gas.^{45,97} Similar results have been obtained in spontaneously breathing rats in which maintenance of normocapnia during hypoxemia diminished the increase in renal vascular conductance observed during hypocapnic hypoxemia.⁵³ Also in rats, Minura et al reported that normoxic, hypocapnic hyperventilation, but not normocapnic hyperventilation, increased renal excretion rates of sodium and water.⁵⁹ In the pioneer work of Ullmann, the natriuretic response to hypoxemia in humans seemed to be blunted by addition of CO₂.⁹⁵

Two distinct mechanisms may account for these effects of hypocapnia on renal hemodynamic and excretory responses to hypoxemia. First, the neural discharge from peripheral chemoreceptors in response to a given level of hypoxemia very much depend on the arterial CO₂ level.^{40,51,82} Whereas hypercapnia increases afferent activity to the vasomotor center, hypocapnia decreases the firing rate of the fibers so that lower levels of hypoxemia are necessary to reach the breakpoint of the stimulus-response curve.⁸² Thus, sympathetic vasomotor outflow and in turn RSNA should be expected to be lower during hypocapnic hypoxemia compared with similar levels of eucapnic and hypercapnic hypoxemia.

Second, hypocapnia may interfere with proximal tubular reabsorption of bicarbonate and sodium. Catalysed by carbonic anhydrase, CO₂ inside tubular cells is hydrated to form carbonic acid, which is then dissociated to hydrogen ions and bicarbonate. The hydrogen ions are exchanged across the brushborder membrane with sodium by the Na⁺-H⁺ antiport, and within the tubular lumen the carbonic anhydrase catalysed process is reversed so that the CO₂ formed by combination of hydrogen ions with filtered bicarbonate can diffuse across the tubular cells to the blood. The net result of these processes is reabsorption of sodium and bicarbonate. However, in the presence of increased respiratory loss of CO₂ causing an increase in peritubular pH, the fractional reabsorption of filtered bicarbonate and sodium is reduced. In humans, the natriuretic response to respiratory alkalosis with or without hypoxemia is associated with a rise in urine pH and an increase in the renal excretion of bicarbonate.^{34,95}

Inhibition of the proximal tubular reabsorption of sodium and bicarbonate would tend to increase the delivery of water and sodium to the distal tubules. Because lithium is reabsorbed in parallel with sodium in the proximal tubules and neither reabsorbed nor secreted in the distal tubules, the renal clearance of lithium can be used to estimate proximal tubular outflow to the thin descending limb of the loop of Henle.^{10,68,70,93} We have used the lithium clearance method in normal subjects to test the hypothesis that acute hypocapnic hypoxemia increases proximal tubular outflow to the same extent as hyperventilation, and that this response is blunted when CO₂ is added to the hypoxic gas to produce normocapnic hypoxemia (Christensen et al, unpublished results). The results are shown in Figure 3. The fractional clearance of lithium increased during hypocapnic hypoxemia and hyperventilation, but remained unchanged during normocapnic hypoxemia. GFR remained unchanged on all study days, and thus fractional proximal tubular reabsorption (1 - (C_{Li}/GFR)) decreased during hypocapnic hypoxemia and hyperventilation, but remained unchanged during normocapnic hypoxemia. The results demonstrate that respiratory alkalosis with or without hypoxemia decreases proximal tubular reabsorption and that this effect is abolished by addition of CO₂ to the hypoxic gas.

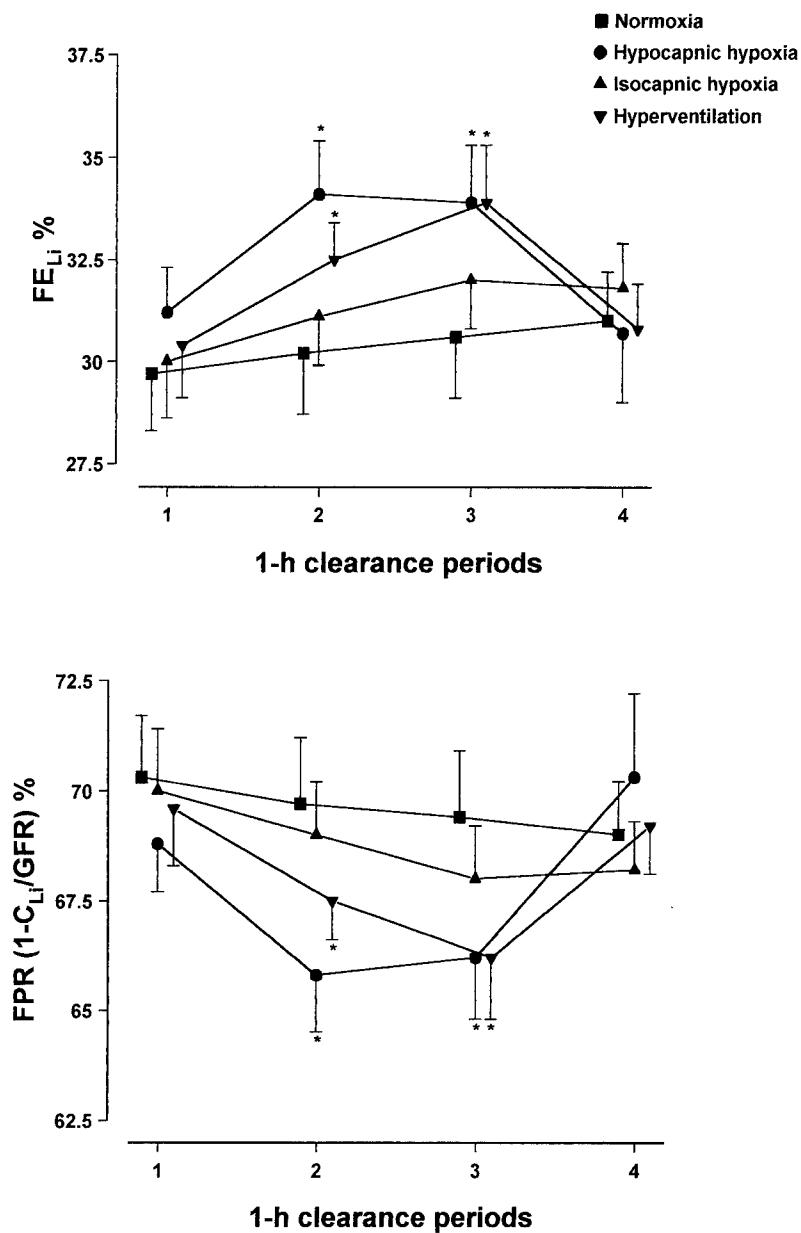


Figure 3 Effects of hypocapnic and normocapnic hypoxia (10% O_2) and normoxic hyperventilation on fractional excretion of lithium (FE_{Li} = renal clearance of lithium/GFR) and fractional proximal tubular reabsorption (FPR) in normal subjects (see text). In each subject, the different gas mixtures were administered during two consecutive 1-h periods on separate study days. Normocapnic hypoxia was achieved by addition of CO_2 to the hypoxic gas. Hyperventilation was adjusted to produce a similar decrease in P_aCO_2 as during hypocapnic hypoxia. Period 1, baseline; periods 2 and 3, experimental periods; period 4, recovery. Values are means with SEM, $N=8$. *, $P < 0.05$ compared with normoxemia (normal breathing of room air through the same tight-fitting face mask as used on the other study days). (Christensen et al, unpublished results).

It remains controversial, however, whether or not the effects of hypocapnia on chemoreceptor sensitivity and proximal tubular reabsorption contribute significantly to hypoxia-induced changes in renal function. In our study, the renal plasma flow increased to a similar extent on the three study days with hyperventilation, hypocapnic hypoxemia and normocapnic hypoxemia (Fig. 2). This suggests that the increase in renal perfusion is related to the increased ventilation *per se* rather than to changes in arterial CO₂ levels. Regarding hypoxia-induced natriuresis, it has been shown that this response may as well occur during normocapnic conditions,⁹⁷ and that the sodium excretion exceeds bicarbonate excretion during respiratory alkalosis produced by hypoxia.^{34,46} Recently, Swenson et al demonstrated that the hypoxia-induced natriuresis in humans correlated positively with the magnitude of the hypoxic ventilatory response but not with bicarbonate excretion,⁹² as would have been expected if the increase in sodium excretion was primarily caused by inhibition of proximal tubular reabsorption of bicarbonate.

Humoral changes during acute hypoxemia

Accompanying changes in circulating levels of renin, aldosterone, atrial natriuretic peptide (ANP) and antidiuretic hormone (ADH) are of main interest in relation to renal function. During the first days in high altitude, resting values of renin decrease in spite of increased norepinephrine levels.^{4,69} During short-term hypoxic exposure, most studies have reported unchanged values of plasma renin activity.^{18,20,21,39,47,92} It has been clearly demonstrated that hypoxemia selectively inhibits aldosterone secretion in adrenocortical cells,⁷⁵ and this effect most likely accounts for the decrease in plasma aldosterone concentration observed during acute hypoxemia.^{20,39,47} Other studies, however, reported unchanged values.^{18,21,92} Acute hypoxemia often increases ANP.^{19,42,47} The responsible mechanisms involve both a direct effect on heart muscle cells² and effects secondary to hypoxia-induced increases in pulmonary artery pressure.^{3,42} Changes in ADH seem to be more unpredictable. First, if hypoxemia evokes nausea, as can be seen with severe hypoxemia, this induces brisk increases in ADH secretion that instantly results in antidiuresis. Also alertness, arousal and anxiety may increase ADH secondary to increases in plasma catecholamines. On the other hand, ADH may decrease during mild and moderate hypoxemia, perhaps due to the increase in intrathoracic blood volume induced by hyperventilation.¹⁸ Taken together, humoral changes during hypoxemia would tend to promote renal excretion of sodium and water, except in conditions where ADH increases to induce a marked antidiuretic response. However, in view of the rapid onset of hypoxia-induced changes in renal hemodynamics and excretory function, the role of humoral changes are not likely to be of major importance.

Summary

The integrated renal response to acute hypoxemia constitutes an example of an interplay of several complex physiological systems. Strikingly, hypoxemia in spontaneously breathing animals and humans does not result in marked renal and visceral vasoconstriction as does asphyxia and apnea, and, in most species, the renal response may include diuresis and natriuresis. Obviously, the primary cardiovascular response to chemoreceptor stimulation is opposed, but the evidence obtained thus far does not allow firm conclusions about the relative importance of the lung inflation reflex, hypocapnia, and of local vascular and tubular effects of hypoxia.

Growing evidence, however, points toward local vascular effects as being the most important factor for hypoxia-induced renal vasodilation, at least in rats.^{54,58,63} Nonetheless, it should be noted that at least two factors when present are likely to govern the overall renal response to hypoxemia. First, activation of the renal-body fluid system secondary to changes in arterial blood pressure is instantly followed by an appropriate change of the renal tubular handling of sodium and water. Second, even small changes in renal blood flow can greatly influence sodium excretion even in the absence of concomitant changes in GFR.²⁶ Although the exact tubular mechanisms remain unsettled, some evidence exists to suggest that drug-induced renal vasodilation causes natriuresis by alterations in medullary hemodynamics and/or renal interstitial pressure secondary to an increase in medullary blood flow.^{17,30} This may decrease fluid and solute reabsorption in the loop of Henle.³⁰ Hypoxemia often causes renal vasodilation that by similar mechanisms may result in natriuresis. However, further studies are needed to investigate the effects of hypoxemia on intrarenal hemodynamics.

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ABSTRACTS OF THE TENTH INTERNATIONAL HYPOXIA SYMPOSIUM

1

PULMONARY EDEMA AT ALTITUDE AND SEA LEVEL FOLLOWING ENDURANCE EXERCISE

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Following endurance exercise (EX), some subjects develop spirometric and gas exchange abnormalities consistent with interstitial pulmonary edema. Chest radiography is a sensitive, non-invasive technique for detection of edema. To determine what radiographic changes suggestive of edema occurred after EX at moderate altitude (ALT) and near sea level (SL), 47 well-trained cyclists were studied (10 at SL and 37 at ~2500–2800m ALT). Over 3–6 hrs cyclists completed a difficult hilly course as rapidly as possible and were compensated based on performance. Before and immediately following EX, PA and lateral chest radiographs (CXR) were obtained. CXR were individually evaluated in random order for signs of edema by a chest radiologist who was unaware of whether the films were obtained before or after EX. CXR were evaluated (0–2 scale) for recruitment of upper lung blood vessels, loss of definition of the vascular markings, hilar blurring, Kerley lines, peribronchial or perivascular cuffing, widening of the fissures, and pleural effusion which were summed to give an edema score. Prior to EX the edema score was 1.69 ± 1.61 (mean \pm S.D.). Following EX this score increased significantly to 2.55 ± 2.04 , $p < 0.05$ by ANOVA. CXR results were not different at ALT vs SL, possibly due to fewer subjects studied at SL. Before EX 8 of 47 subjects had edema scores of >3 , whereas after EX 16 of 47 CXR had scores >3 . Peribronchial cuffing was the most frequent new abnormality after EX. These data suggest that radiographic changes of early pulmonary edema occur frequently following high intensity exercise and are influenced little by altitude.

2

EFFECTS OF THE SIMULATED ALTITUDE TRAINING ON AEROBIC WORK CAPACITY IN THE HIMALAYAN CLIMBERS

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This study was designed to elucidate the physiological effects of the simulated altitude training to respirocirculatory and muscle metabolic function in the Himalayan climbers. Six male subjects aged 19–43 (mean, 34) years old were studied. For altitude training, a submaximal pedalling on Monark ergometer was performed for 12 weeks with once per week at hypobaric simulator in 5,000 m, 6,000 m, 6,500 and 7,000 m before departure for climbing. For VT- $\dot{V}O_2$, $\dot{V}O_{2\max}$, SaO_2 and StO_2 in M. vastus lateralis determination used by NIRS (PSA-III) before, after training and climbing, incremental maximal pedalling work were performed at 4,000 m. SaO_2 during work indicated a tendency of increase after training and a significant increase after climbing. StO_2 during work also showed a tendency of increase after training and a significant increase after training, although VT- $\dot{V}O_2$ and $\dot{V}O_{2\max}$ were almost the same with the value before, after training and climbing. $PCr/PCr+Pi$ ratio after 10 min right leg lift work in magnet capsule at supine measured by NMR indicated a tendency of increase from average 0.52 before training to 0.56 and 0.62 after training and climbing, whereas time constant at recovery from the work showed a tendency of decrease from average 48.2 before training to 44.9 and 40.7 after training and climbing. From these results, it might be suggested that the improvement in oxygen transporting system by this simulated training would be effectively contributed to prevent for mountain sickness.

Supported by a grant from Ministry of Education, Culture & Sports of Japan

ABSTRACTS

3

INHIBITORY EFFECT OF HYPOXIA ON HUMORAL IMMUNITY OF RATS*

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To study the effect of hypoxia on humoral immunity function of rat and *Ochetona curvirostris* (pika), the specific antibody production to novel antigen IgG and immunoresponse to sheep red blood cell (hemolysis forming) were measured. The results show: Hypoxia at altitude of 5km and 7km for 10d resulted respectively in 10.3% (P<0.05) and 21.9% (P<0.05) decrement in hemolysis forming in rats, as compared with the control group kept at 2.3 km. When the rats were secondarily immunized and kept at the same hypoxia for 10d, the reduction in hemolysis forming was 4.2% (P<0.05) and 4.6% (P<0.05) for the two respective altitudes. However those effects were not found in the pikas. When the rats were immunized two days before hypoxia, hypoxia of 5km for 5d and 8d failed to suppress hemolysis formation. Intracerebroventricular (icv) injection of CRF (1.0 µg/rat), decreased hemolysis formation and the production of IgG by 8.6% (P<0.05) and 14.0% (P<0.05) respectively, but intraperitoneal (ip) injection of CRF (1.0 µg/rat) had no effect. However, icv injection of CRF receptor antagonist (α-helical CRF(9-41), 50 µg/rat) prior to 7km hypoxia caused a hypoxia-induced suppression of IgG production from 24.2% to 12.1% (P<0.05). Adrenalectomy in rats lowered hemolysis formation by 6.6% (P<0.05). The above results demonstrated that hypoxia suppresses humoral immunity function and alters initial antigen processing probably through increase of CRF in CNS.

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4

THE EFFECT OF HYPOXIA ON CELLULAR IMMUNE FUNCTION OF NEONATAL RATS*

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The effect of hypoxia on the cellular immune function of neonatal rats in age of 14 days as well as on the levels of ACh, catecholamine in spleen were studied. When the animals were exposed to 5km simulated altitude in hypobaric chamber for 5 days which resulted in a 43.4% decrement in DNA contents in spleen lymphocytes and a 13.2% decrease in lymphocyte proliferation. Similar suppression of the immune function and DNA in baby rats exposed to 7km for 24h was noted as well, decreased by 39% and 19.8% respectively. The suppressive effects of hypoxia of 7km 24h on DNA contents were partly blocked when rats were pretreated intracerebroventricularly with DSP-4 one day before hypoxia. The levels of catecholamine in spleen were increased, meanwhile the levels of ACh were decreased after exposure to 7km for 24h. These observations indicate that hypoxia suppresses cellular immune function of neonatal rats and sympathetic-parasympathetic nervous system might be involved.

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5

NOREPINEPHRINE REGULATION OF T-LYMPHOCYTE PROLIFERATION OF RATS DURING ACUTE HYPOXIA*

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Interaction between the immune and neuroendocrine systems during hypoxia has not been noted. In the present study, the role of NE in the immunoregulation in rats during simulated hypoxia in hypobaric chamber was examined. It was found that hypoxia of 7km for 24h inhibited T-lymphocyte proliferation by 41%. Hypoxia for 7d and 20d of 5km altitude reduces T-lymphocyte proliferation 34% and 60% respectively. Intracerebroventricular (icv) injection of 5 × 10⁻⁶ mol/L NE (2 µl) decreased T-lymphocyte proliferation by 29%. ICV injection of phenolamine 25 µg/rat prior to hypoxia of 7km for 10h attenuated hypoxia-induced suppression of T-lymphocyte proliferation from 42% to 21%. In addition, hypoxia of 7km for 10h increased CRF levels in blood and catecholamines contents in hypothalamus. An increased circulate CRF level was also noted after NE injection (icv) of 5 × 10⁻⁶ mol/L 2 µl. Rats were exposed to hypoxia at 7km for 10h, dispersed spleen lymphocytes incubated with certain dose of CRF in vitro. T-lymphocyte proliferation decreased in dose-dependent with increasing CRF concentration. These findings suggest that hypoxia inhibits T-lymphocyte proliferation through an immune inhibition action by modulation of CRF-NE.

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*Supported by NNSFC

6

WOMEN AT ALTITUDE: CHANGES IN CARDIAC OUTPUT AND PULMONARY DIFFUSING CAPACITY DURING EXERCISE*

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To determine cardiac output (\dot{Q}_c , ml/min/m²) and pulmonary diffusing capacity (DL, ml/min/mmHg/m²) in women at high altitude (HA), we studied 17 healthy women (age 21.7 ± 0.5 yrs) by a rebreathing technique at rest and during steady state exercise at 50% of the maximal work load achieved at sea level (SL). Studies were repeated at the same absolute work load after 10 days of stay at Pikes Peak (4,300 m). HR=heart rate (bpm) during rebreathing at 30 breath per min. $\dot{V}O_2$ =O₂ uptake (ml/min/m²). SI=stroke index (ml/m²). Ca-vO₂=arterio-venous O₂ content difference (ml/dl). Mean±SE. * p <0.05 vs. SL.

	Sea level		4,300 m	
	Rest	50% max	Rest	50% max
HR	97±4	149±5	102±2	141±2 *
VO ₂	134±7	756±20	143±4	800±21
DL	12.6±0.5	18.9±1.0	17.2±0.6 *	21.6±0.9 *
Qc	3.71±0.11	7.89±0.35	3.29±0.16	7.60±0.23
SI	39±2	53±3	32±2 *	47±2 *
Ca-vO ₂	4.0±0.2	10.6±0.7	4.5±0.2	10.7±0.4

At HA, DL was significantly elevated at a given \dot{Q}_c due to a lower alveolar PO₂; this response is similar to previous reports in men. SL remained low but the relationship of \dot{Q}_c to VO₂ had returned to baseline due to a higher HR. Normalization of \dot{Q}_c occurred within 10 days at HA, earlier than had been previously studied in men.

Supported by DWHRP contract DAMD 17-95-C-5110 and the American Heart Association.

COUGH RECEPTOR SENSITIVITY AND DYNAMIC VENTILATORY RESPONSE TO CARBON DIOXIDE IN MAN ACCLIMATISED TO HIGH ALTITUDE

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Cough receptor sensitivity, measured by challenge with inhaled citric acid, and dynamic ventilatory responses to inhaled CO₂ both increase with acclimatisation to high altitude (Barry et al. *Thorax* 1995;50:A54; Collier et al. *Journal of Physiology* 1995;487:136P). We report on the relationship between the responses to these two stimuli. Twenty members of the British Mount Everest Expedition were studied after 7 or more days acclimatisation to 5430m. Ethical committee approval was obtained. Subjects inhaled increasing concentrations of nebulised citric acid, using a standardised breathing pattern. The cough threshold was defined as the concentration of citric acid which provoked cough, provided the next concentration also provoked cough. In a separate experiment, subjects breathed small volumes of 10% CO₂ either early or late in inspiration, during moderate (60w) exercise. Both early (CO₂E) and late (CO₂L) stimuli increased end tidal CO₂ concentrations by similar amounts, average (SEM) 4.20(4)mmHg for CO₂E and 4.00(5)mmHg for CO₂L. Mean ventilation increased by 17.8(2.1)lmin⁻¹ with CO₂E, and by 10.5(1.2)lmin⁻¹ with CO₂L. Overall, the [log(citric acid)] cough threshold was inversely related to the ventilatory response to the CO₂E stimulus ($r=0.5$, $p=0.037$ ANOVA), but was not related to the ventilatory response to the CO₂L stimulus. There is considerable intersubject variability in cough receptor sensitivity, which is unexplained. This study suggests a relationship between dynamic CO₂ ventilatory response and cough receptor sensitivity. In man, dynamic CO₂ sensitivity is probably mediated by peripheral chemoreceptors. Our results support a link between their stimulation and the airway cough receptor.

INSPIRATORY FLOW AT ALTITUDE

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A number of studies have shown changes in expiratory flow at altitude (Pollard et al. *Thorax* 1996;51:175-8), but corresponding changes in inspiratory flow have not been described. We studied members of the British Mount Everest Medical Expedition at sea-level, on arrival at Everest Base Camp, Nepal (5,300m) and again after a further ten days acclimatisation to altitudes of greater than 5,000m. Ethical committee approval and informed consent were obtained. Subjects recorded flow volume curves using a fixed orifice turbine spirometer (Microloop, Micro Medical Ltd, Kent, UK). Each measurement was made in a large research tent, and the best of three measurements were recorded. Expressed as percentage change from sea-level, peak inspiratory flow (PIF) and inspiratory flow at 50% vital capacity (I₅₀) both nose on the first visit to base camp, PIF by 26.9% (54.32), I₅₀ by 27.9% (56.26), and on the second visit, PIF by 28.0% (27.3), I₅₀ by 25.0% (21.8) at the second. The considerably smaller variation in the second visit is at least partly explained by the presence of one outlier in the first visit data, who improved his peak inspiratory flow by 250%. Both PIF and I₅₀ were significantly higher at altitude compared to sea-level, but there were no significant differences between results obtained on the first and second base camp visits. Ambient temperature in the research tent varied from 10°C to 25°C, and this data has not been corrected for changes in temperature. However, calculated corrections would be smaller than the degree of observed changes in spirometric values. This study confirms that, like expiratory flow, inspiratory flow rises on ascent to altitude, probably due to changes in gas density decreasing resistance to respiratory gas flow.

URINARY LEUKOTRIENE E₄ LEVELS ARE NOT INCREASED IN HIGH ALTITUDE PULMONARY EDEMA

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To test the hypothesis that lipoxigenase products contribute to increased capillary permeability in high-altitude pulmonary edema (HAPE), we measured urinary leukotriene E₄ (LTE₄) excretion in HAPE-susceptible subjects during exposure to real and simulated altitude. Urinary LTE₄ was measured after extraction on Sep-Pak cartridges and HPLC purification system by a specific ELISA. At 4,559 m, 2 subjects developed radiographic evidence of HAPE on the second and 3 on the third day of exposure. In these 5 subjects, mean levels of urinary LTE₄ excretion were not significantly increased on day 2 (329 ± 137 (SD) ng/24h) or day 3 at high altitude (426 ± 229), when compared to control measurements at low altitude (311 ± 113). During exposure to a simulated altitude of 4,000 m over 24 hours, urinary excretion of LTE₄ (389 ± 71 ng/24h) in 7 HAPE-susceptible subjects was not significantly different from values obtained during their normoxic control period in the chamber (402 ± 51) nor from values obtained in 5 non-susceptible controls in normoxia (377 ± 141) and hypoxia (420 ± 136). These data suggest that leukotriene-mediated vascular injury does not occur prior to HAPE nor in early HAPE.

ACUTE MOUNTAIN SICKNESS IN WOMEN WITH REPEAT EXPOSURE TO 4300 M. P.B. Rock, S.R. Muza, C.S. Fulco, B.A. Beidleman, P. Ondrus, T.P. Lyons and A. Cymerman. US Army Resch. Inst. Environ. Med. Natick, MA, USA 01760.

Background. It is widely believed that most unacclimatized individuals have a specific susceptibility to acute mountain sickness (AMS) which causes them to experience similar symptoms during each sojourn to high altitude. Except for one reported study (Robinson et al. *Aerospace Med.* 42:706-8, 1970), the belief is based on anecdotal evidence. In that study, nine of 11 volunteers (82%) had nearly reproducible AMS symptom scores during two, 36 h exposures to 4300 m simulated altitude in a hypobaric chamber. We tested the hypothesis that women like men, would experience consistent susceptibility to AMS with repeated exposure to high altitude. **Design.** Ten young, healthy women were exposed to a 4300 m simulated altitude in a hypobaric chamber for 30 h on three occasions separated by ≥ 14 days between exposures. Symptoms of AMS were assessed using the Environmental Symptoms Questionnaire (ESQ-III). **Results.** All of the women were without symptoms of AMS during at least one exposure. Fifty percent experienced AMS on 2/3 exposures, 20% experienced AMS on 1/3 exposures and 30% did not experience AMS during any of the three exposures. **Conclusions.** Unlike men, unacclimatized women do not appear to experience AMS consistently during repeated exposure to high altitude. The basis for this intra-individual variability in women is not known, but may be related to effects of ovarian steroid hormone fluctuations in different phases of the menstrual-cycle.

ABSTRACTS

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SECOND GENERATION LOWLAND TIBETANS LOSE LESS AEROBIC POWER AT ALTITUDE THAN CAUCASIANS

Bengt Kayser, Mauro Marzorati, Tiziano Binzoni, Bruno Grassi, Claudio Marconi, and Paolo Ceretelli, ITBA-CNR, Via Ampère 56, 20131 Milano, Italy; Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland. In order to test the hypothesis that Tibetans possess inborn traits allowing them to better perform at altitude than lowlanders, 8 volunteer second generation Tibetan men (T) (20±2yr, 170±8cm, 57±9kg), born and living at 1500 m without previous high altitude history were exposed to 5050m for 4 wk (CNR-pyramid, Lobuche, Nepal). To that aim their peak aerobic power ($\dot{V}O_{2\text{max}}$) was compared to that of a lowlander control group (L, n=7, 30±4yr, 183±8cm, 83±13kg). Baseline $\dot{V}O_{2\text{max}}$ (1300m) of T was 38±5ml/min/kg and that of L (60m) 41±6ml/min/kg (NS). After one wk at 5050m $\dot{V}O_{2\text{max}}$ was reduced to 75% in T and 57% in L. By the end of 4 wk $\dot{V}O_{2\text{max}}$ attained 93% of control in T whereas in L it only reached 67%. The loss of $\dot{V}O_{2\text{max}}$ for L was accompanied by a marked decrease in SaO_2 and maximum heart rate (HRmax) that did not recover over 4 wk (from 96±1% to 77±7% and from 180±15/min to 154±8/min, respectively). By contrast, upon altitude exposure T showed only moderate changes of SaO_2 and HRmax (from 96±1% to 82±6% and from 188±13/min to 179±9/min, respectively). After 4 wk the ventilatory equivalent ($\dot{V}E/\dot{V}O_2$) (-80), R (-1.15) and PETO_2 (-58 Torr) at $\dot{V}O_{2\text{max}}$ were similar between T and L. The different changes of SaO_2 and PETO_2 in T and L are compatible with a much smaller A-aDO₂ gradient in T than in L. This could be due to differences in lung diffusion characteristics, V/Q distribution, or oxygen affinity for hemoglobin. In conclusion: 1) at high altitude T loose initially less and regain over 4 wk more aerobic power than L; this may be related to more efficient gas exchange at the lung and at the periphery; 2) T seem to preserve a greater cardiac output at altitude than L; 3) the described differences in T compared to L appear to be inborn because present also in T who were never exposed to high altitude.

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INTERACTION BETWEEN BREATHING PATTERN AND AUTONOMIC MODULATION DURING ACUTE HYPOXIA INDUCED BY SIMULATED ALTITUDE

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To assess the relationship between different breathing patterns and autonomic cardio-vascular modulation (AutCVM) during acute adaptations to altitude induced hypoxia, we measured relative changes in ventilation (VE), oxygen saturation (%SaO₂), RR interval and diastolic blood pressure (DBP, Cohn®) at Albuquerque level (SL, 1500m) and after acute (1h) hypobaric hypoxia (HA, equivalent to 5000m, pressurized chamber) in 10 western yoga practitioners (YP, age 38±3yr) and 9 controls (CTL) of similar age. While breathing spontaneously (Fbr), at 15b/min (Cbr) and during "complete yoga breathing" (slow diaphragmatic+thoracic breathing, ~4b/min) in YP or simple slow breathing in CTL (Ybr), AutCVM was assessed by spectral analysis (sympatheto-vagal balance: ratio of low-to-high frequency fluctuations, LF/HF). At SL, %SaO₂, VE and AutCVM were similar in both groups. HA decreased RR interval (from 879±43 to 770±39, p<0.01) and increased LF/HF (from 1.6±0.5 to 3.2±1.1, p<0.05) in CTL during Fbr and Cbr, indicating sympathetic activation; these changes were blunted in CTL during Ybr. In YP no autonomic changes were observed. DBP and sympathetic modulation to the vessels remained unchanged in both groups. Compared to SL, HA increased VE during Fbr, Cbr and Ybr, but decreased %SaO₂ by 17%, 14% and 14%, respectively (p<0.0001) in CTL; in YP, HA increased VE only during Fbr, and %SaO₂ decreased only by 12%, 9% and 8% (all p<0.01 vs CTL). Conclusion: well performed Ybr maintains high blood oxygenation without increasing VE (i.e. is a more efficient breathing) and reduces sympathetic activation during HA. Support: IRCCS S.Matteo and C.Mondino, Italy, NMHEMC Res.Foundation

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CARDIOVASCULAR AUTONOMIC MODULATION:

EFFECTS OF HYPOXIA IN PARKINSON'S DISEASE (PD)
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Oxygen chemosensitivity is decreased in PD and disorders of autonomic function have been reported in this disease. We examined autonomic control of heart and arterial vessels by spectral analysis of RR intervals and blood pressure variability and efferent sympathetic and vagal modulation of the heart and vessels with autoregressive spectral analysis in 5 healthy and 7 age matched PD males (Hoehn-Yahr disability scale 2.0-2.5), in normoxia and while breathing 11% O₂ supine at sea level. The rebreathing technique (RBHT), in which O₂ concentration fell (isocapnic hypoxia) with time (usually 5-6 min.), until PETO₂ reached 40-35 mm Hg was also used. No significant differences in any parameters between the study groups were found during normoxia or while breathing 11% O₂. At the end of RBHT there were significant increases in low-frequency power in systolic and diastolic pressures (LF-SYS, LF-DIAS) in controls. In PD no significant changes in LF-SYS, LF-DIAS occurred consistent with impaired autonomic control of blood vessels; this may also reflect altered chemosensitivity in PD. Support: NMHEMC Research Foundation, IRCCS, S. Matteo, Istituto C. Mondino, Univ. of Pavia Italy.

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AUTONOMIC CHANGES DURING PROGRESSIVE HYPOXIA: EFFECT OF INTERVAL HYPOXIC TRAINING

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Interval hypoxic training (IHT) was proposed as a technique able to increase the hypoxic ventilatory response (HVR); its effects on cardiovascular autonomic control are unknown. To assess this point we recorded ECG (RR interval), noninvasive systolic blood pressure (SBP, Finapres®), ventilation (VE), PaO_2 and PaCO_2 , during progressive isocapnic hypoxia (PH, from 20% to 5-7%) before and after 14 days of: a) IHT (3-4 periods of 7-minute PH in 1 hour, for each day) in 12 healthy male subjects (IHT-group, age 27±2 yr); b) normal breathing into a spirometer in 6 (C-group) age-matched male controls. HVR was estimated by the hyperbolic relation between PaO_2 and VE (shape factor A). Autoregressive spectral analysis was used to characterise low- (sympathetic) and high-frequency (vagal, respiration-related, HF) fluctuations. IHT increased shape factor A in the IHT-group from 268±59 to 984±196 L/mmHg (p<0.003) but not in the C-group (from 525±180 to 808±245 L/mmHg, p=ns). Before IHT, PH decreased mean RR, RR variability (70±1 to 39±5ms, p<0.05) and HF power to a similar extent in both groups. After IHT, RR still significantly decreased, but the decrease in RR variability and HF power was no longer significant (from 75±10 to 63±10ms, p=ns) in the IHT-group. No significant changes were observed in mean SBP and SBP spectral components. No changes were observed in the C-group. The IHT-induced increase in HVR is associated to reduced vagal withdrawal and/or reduced sympathetic activation during PH; this might be beneficial by reducing the sympathetic-dependent oxygen demand during hypoxia. Support: IRCCS S.Matteo and C.Mondino, Italy, NMHEMC Res. Foundation

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OSMOREGULATION IN MAN AT HIGH ALTITUDE: RENAL AND ENDOCRINE EFFECTS OF A HYPERTONIC SALINE LOAD.

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Water balance - regulated through changes in plasma osmolality (p-OSM) and plasma arginine vasopressin concentration (p-AVP) - is often changed at high altitude (HA). This study investigated HA-induced changes of the relationship between p-OSM, p-AVP and renal excretion of sodium and water. Eight male volunteers were investigated at sea level (SL) and again at 4,559 m. At HA p-AVP and p-OSM were measured every morning on days 1-3 and 6-8. At SL and on day 6 at HA water diuresis was induced by a 500 ml water load which was continuously sustained. After 3 hours a hypertonic saline load (5% saline, 3.6 ml/kg body weight) was administered intravenously over 1 hour. Morning values of p-OSM increased from 291±1 at SL to a maximum of 296±1 mOsmol/kg on day 7 at HA ($p<0.01$). p-AVP decreased from 1.24±0.2 at SL to a minimum of 0.4±0.1 pg/ml on day 7 ($p<0.01$). In response to the sodium load, p-OSM increased at SL from 295±1 to 298±1 ($p<0.01$) and at HA from 297±1 to 302±1 mOsmol/kg ($p<0.01$). p-AVP increased from 0.2±0.1 to 0.6±0.1 pg/ml at HA ($p<0.01$). 60 min after the sodium load, urine flow rates at SL and HA decreased by 42% compared with pre-infusion values ($p<0.01$). After 120 min, sodium excretion rates increased to the same extent by 20% (SL, $p<0.01$) and 16% (HA, $p<0.01$). In conclusion, the present results demonstrate that p-AVP is reduced at HA despite an increased p-OSM. Also, the results indicate that the AVP response to an osmotic stimulus is not abolished at HA as has been reported by others (Blume et al. JAMA 252: 1580, 1984 and Ramirez et al. Aviat Space Environ Med 63: 891, 1992). The antidiuretic and natriuretic responses to a hypertonic saline load remained unchanged at HA.

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THE ENERGY COST OF SPORT ROCKCLIMBING IN ELITE PERFORMERS

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Dedicated to our friend John Sutton.

The increase in the number and difficulty of rockclimbing first ascents can be attributed to developments in sport climbing. However, the physiological factors related to sport climbing remain undefined. The aim of this study was to investigate the energy requirements of sport climbing for five elite sport climbers (28.4 ± 1.8 yrs, 177.2 ± 2.3 cm, 64.9 ± 2.3 kg, 8.6 ± 0.79 % body fat; mean ± SEM).

Climbers were assessed using an indoor vertical treadmill fitted with artificial rock hand/foot holds. Climbing velocity (hence average power output) was incremented until voluntary fatigue. This assessment elicited a $\dot{V}O_{peak}$ and heart rate of 43.36 ± 2.39 ml·min $^{-1}$ and 189 ± 2 bpm, respectively with blood [lac] 9.4 ± 0.7 mmol $^{-1}$. On a separate occasion the climbers performed an outdoor rockclimb (length 24.52 m, grade 5.11a) at a "comfortable" climbing speed. Cardiorespiratory parameters were measured using a telemetry system (wt. 3 kg) and blood lactate collected at rest and within 3 min of completing the climb. The duration of the climb was 6 min 31s ± 31s and heart rate at 1, 3, and 5 min and at the end of the climb were 143 ± 6, 146 ± 6, 156 ± 5, and 156 ± 8 bpm, respectively. After 3 and 5 min climbing $\dot{V}O$ was approximately 67 % of climbing $\dot{V}O_{peak}$. Blood lactate increased from 1.1 ± 0.2 at rest to 4.2 ± 0.6 mmol $^{-1}$ at the end of the climb.

The results suggest that for elite climbers, moderately graded sport climbs of several minutes duration require a significant portion of climbing $\dot{V}O_{peak}$. The increased blood lactate suggests a contribution by the anaerobic energy system possibly related to repeated isometric contractions of the forearm muscles.

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BLOOD PLATELET COUNTS IN YOUNG BOLIVIAN AIRMEN VISITING THE ANDES

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Previous studies of peripheral blood platelet counts at altitude have yielded conflicting results. This study was designed to investigate the effects of hypobaric hypoxia on platelet numbers in longterm highland residents and lowlanders following ascent to high altitude. We studied 175 volunteers from the Bolivian Air Force stationed at El Alto (4200m) for at least 1 year, and 105 volunteers, matched for age and sex, living in Santa Cruz (600m). We also studied a lowland population 48 hours and 1 week following ascent to 3600m, and again 2 weeks after their return to 600m. Platelet counts were measured immediately after sampling and serum samples were stored for thrombopoietin assays on return to the UK. Thrombopoietin has been demonstrated to be the primary regulator of platelet production in man. We found a significant difference in platelet counts between the low altitude (mean $271 \times 10^9/l$) and high altitude groups (mean $471 \times 10^9/l$). In the lowlanders ascending to 3600m, mean platelet counts were $367, 398$ and $251 \times 10^9/l$ after 48 hours & 1 week at high altitude and 2 weeks after return to 600m, respectively. Thrombopoietin assay results are awaited. We postulate that the initial rise in platelet count may be due to haemocencentration but the sustained elevation of platelets in young Bolivian airmen at altitude is likely to be under hormonal control, perhaps mediated by thrombopoietin. This increase in platelet count may contribute to the pathological finding of thrombosis associated with high altitude pulmonary and cerebral oedema and may also be of importance in the aetiology of high altitude stroke. Supported by a grant from the University of Liverpool, UK.

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ACETYLSALICYLIC ACID FOR PROPHYLAXIS OF HIGH ALTITUDE HEADACHE

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Headache is known to be the most predominant symptom in acute mountain sickness (AMS). Non-steroidal anti-inflammatory drugs like ibuprofen are effective for therapy of high altitude headache (HAH). Nevertheless, one of the most commonly used substance for treatment and/or prophylaxis of headache might be acetylsalicylic acid (ASA). However, no information exists regarding its preventive effectiveness for HAH. Therefore, ASA was tested for prophylaxis of HAH in a randomized, double-blind trial. 29 volunteers were randomized to the placebo group (PG; 8 men, 6 women, mean age: 38 yrs) and to the acetylsalicylic acid group (AG; 9 men, 6 women, mean age: 38 yrs). They were transported from Innsbruck (600 m) to an altitude of 3460 m and stayed there for 24 hrs. Tablets (placebo, ASA 320 mg) were administered 3 times within 4 hr intervals, beginning 1 hr before arrival at altitude. Headache scores (0-4), arterial oxygen saturation (SaO_2), heart rate (HR) and blood pressure (BP) were determined repeatedly. A 2-min step test and blood gas analyses were performed before and during the altitude sojourn.

7 subjects (4 men, 3 women) out of the PG ($n=14$) and only 1 (woman) out of the AG ($n=15$) developed HAH (headache scores, PG: 1.86±0.69, AG: 3) between 3 and 10 hrs after arrival at altitude ($p=0.014$, Fisher's exact test). SaO_2 values 3 hrs after arrival at altitude significantly predicted the subsequent development of HAH. Mean SaO_2 values did not differ between both groups, however, subjects of the AG tolerated lower SaO_2 (83% vs 88%) without appearance of HAH. Postexercise HRs (min $^{-1}$) between Innsbruck and altitude differed more in the PG (116±14, 142±13) than the AG (118±10, 134±7) ($p=0.013$, ANOVA for repeated measures). Besides, these HR differences were closely correlated to the severity of HAH ($r=0.60$, $p=0.001$).

Since the efficacy of ASA was not attributed to improved oxygenation, it might have reduced hypoxia mediated sympathetic activation by cyclooxygenase inhibition. Acute hypoxia augments prostaglandin levels, which contribute to peripheral and cerebral vasodilation induced in hypoxia. Sympathetic activity is elevated during acute hypoxia supporting cardiac output and blood pressure to offset local peripheral vasodilation. Prostaglandins may also enhance nociception by action at the terminals of sensory neurons. Thus, ASA might have reduced both hypoxic vasodilation and hyperalgesia, resulting in lower sympathetic activity and decreased incidence of HAH.

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NITRIC OXIDE MODULATES HYPOXIA INDUCED INCREASES IN LUNG WATER IN RATS

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High altitude pulmonary edema (HAPE) is a well recognized disease but the exact pathophysiology mechanism has yet to be delineated. Pulmonary hypertension, leading to increased hydrostatic pressure causing capillary leak, is one proposed mechanism. If pulmonary hypertension is the major causative factor in HAPE, then treatment or prophylaxis with a selective pulmonary vasodilator such as nitric oxide (NO) should attenuate or prevent increases in lung water. The objective of this study was to determine if NO would decrease or prevent the increases in lung water in rats exposed to high altitude. Rats acclimatized to the Calgary altitude of 1000 meters (665 torr) were exposed to a simulated altitude of 5450 meters (380 torr). These rats showed 14.0% increases in lung water at 48 hrs ($p < 0.005$) and 19.8% at 72 hrs ($p < 0.001$) when compared to their controls (at 665 torr). A prophylaxis group received 20 ppm NO for the entire 72 hrs at high altitude. This group showed an 11.2% decrease in lung water ($p < 0.05$) when compared to their untreated hypoxic controls. A treatment group received 20 ppm NO for 4 hrs, starting 48 hrs after exposure to altitude. This group showed no decrease in lung water when compared to their hypoxic controls. These data suggest that NO modulates hypoxia induced increases in lung water in rats. NO could be used as an effective agent for prophylaxis of HAPE or as a treatment modality.

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INDIVIDUAL VARIATION IN RESPONSE TO ALTITUDE TRAINING

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Earlier studies by our group have indicated that living at high altitude (2500m), combined with training at low altitude (1250m) (i.e. live high-train low), results in significantly greater improvements in $\dot{V}O_{2\text{max}}$ and performance over equivalent sea level training. Although improvements in group responses are clear (mean \pm SD 5K run time 14.1 ± 36 sec; $\dot{V}O_{2\text{max}}$ 2.5 ± 2.8 ml/kg/min), individual responses display a wide variability (range: $\Delta 5K$ time -112 to 55 sec, $\Delta \dot{V}O_{2\text{max}}$ -3.2 to 8.7 ml/kg/min). To determine the factors which contribute to this variability, 39 runners (27 M, 12 F) were divided into responders and non-responders to altitude training - based on the change in sea level 5K time before and after 4 weeks of living at altitude (2500m). The acute increase in erythropoietin after one night at 2500m ($\Delta[\text{EPO}]$) was examined as an index of the acclimatization response; and the decrease in running velocity during interval training at altitude ($\Delta \text{int vel (SL to Alt)}$) as an index of the impact of altitude on training capability.

Results:

	Non-responders (n=15)	Responders (n=17)	
$\Delta 5K$ time < 0 sec improvement		$\Delta 5K$ time > 15 sec improvement	
$\Delta 5K$ time	-24.0 ± 16.2 sec	36.6 ± 12 sec	$P < 0.01$
$\Delta \dot{V}O_{2\text{max}}$	0.5 ± 2.1 ml/kg/min	4.2 ± 2.8 ml/kg/min	$P < 0.01$
$\Delta[\text{EPO}]$	4.8 ± 2.4 ng/ml	6.5 ± 3.3 ng/ml	$P < 0.05$
$\Delta \text{int vel (SL to Alt)}$	-0.5 ± 0.3 m/sec	-0.1 ± 0.5 m/sec	$P < 0.05$

We conclude that responders to altitude training, compared to non-responders, demonstrate greater increases in $\dot{V}O_{2\text{max}}$, greater increases in $\Delta[\text{EPO}]$ after one night at altitude and smaller reductions in interval training velocity at altitude. Individual assignment of living and training altitudes, based on the erythropoietic and training velocity responses to acute altitude exposure, may minimize the number of non-responders to altitude training.

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INFLUENCES OF HYPOXIA ON CRF STIMULATING cAMP FORMATION IN CULTURED ANTERIOR PITUITARY CELLS OF RAT *IN VITRO*

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To investigate effects of hypoxia on CRF, AVP and NE stimulating the formation of cAMP in cultured pituitary cells of SD rat. CRF rapidly stimulated the formation of cAMP in cultured anterior pituitary cells (the concentrations of CRF were 10^{-10} , 10^{-9} , 10^{-8} pmol, cAMP increased to 188%, 657% and 738%, respectively). The cAMP levels of medium rapidly increased to 431%, 876%, 976%, respectively. The increased cAMP levels correlated with the doses of CRF. The intracellular and extracellular cAMP of cultured cell were not changed by AVP ($P > 0.05$). The concentration of intracellular cAMP was decreased to 10^{-7} nmol, $82\% \pm 10^{-6}$ nmol: 65% (b), NE, but the content of extracellular cAMP was change ($P < 0.05$). Hypoxia decreased the stimulating effects of CRF on the cAMP formation in cultured anterior pituitary cells. 10^{-8} nmol CRF reduced intracellular cAMP of cultured cells to 51% under 8% oxygen.

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HYPOXIC VENTILATORY RESPONSE AT DIFFERENT ALTITUDES

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Increase in Minute Ventilation (MV) is one of the first mechanism of high altitude acclimatization. Different increases in Respiratory Rate (RR) and in Tidal Volume (TV) in accordance with the altitude and the length of the exposure have been described. AIM to assess MV, RR, TV in lowlanders at different altitudes. METHOD: 6 subjects (3F:3M, mean age 39.9 (%), measured MV at sea level, 3500m, 4240m and 5050m during the trekking to Pyramid Lab in Nepal, by means of a sprometer (Spiroflow Schiller). SaO₂ was also measured by means of a finger probe/pulse 503, Criticare Systems Inc). All tests were performed at rest, at the same hour, during the 1st, 2nd, 3rd day of stay at the same altitude. RESULTS expressed as % of sea level value. ANOVA and paired t test were used for statistical evaluation. MV 3500m: 130(37), 116(24), 124(14); 4240m: 116(25), 120(29), 136(21); 5050m: 173(34); 171(42); RR 3500m: 113(20), 115(19), 123(20), 4240m: 113(11), 106(7), 116(11); 5050m: 143(43), 121(19); TV 3500m: 111(16), 100(17), 103(14); 4240m: 104(15), 114(26), 116(18); 5050m: 123(22), 139(19); SaO₂: 3500m 92, 92, 92.5; 4240m: 86.2, 86.6, 88.6; 5050m: 84, 81.2. A significant increase of MV was detected at each altitude compared to sea level between 3500m and 5050m and between 4240m and 5050m. During the stay at the same altitude the only statistically significant value was measured at 3rd day at 4240m. No difference was found between the increase of RR and TV. The decrease of SaO₂ was significantly correlated with the increase of MV. Even with the little number of subjects, we tried a comparison between males and females. Females have a significantly higher increase of RR compared to TV during the first days at HA. Males have a significantly higher increase of TV compared to RR only at 5050m. Supported by ZENECA

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RESPIRATORY HEALTH, INDOOR AND OUTDOOR POLLUTION IN RESIDENTS AT LOW AND HIGH ALTITUDE IN NEPAL: DESCRIPTION OF THE STUDY AND PRELIMINARY RESULTS.

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Indoor and outdoor pollution can affect respiratory health. AIM to investigate respiratory health and exposure to pollution in samples of residents at different altitudes in Nepal. METHOD We studied 102 Nepali subjects: history, clinical examination, exhaled CO, SaO₂%, respiratory function, sputum collection for microbiology and 6hrs monitoring of benzene exposure. All subjects were not smokers, and had no clinically relevant respiratory illness either in the past. Subjects were divided in 3 groups (Kathmandu=K, Namche Bazar 3500m=N Periche 4240m=P); each group was divided in subgroups according to exposure to either environmental or indoor pollution: K exposed: 12 "tampa" drivers; K not exposed: 10 employers, K children: 10 residents in the city and 10 residents in a rural village. N and P exposed: 10 women spending many hours in the kitchen, N and P not exposed: 10 porters, N and P children: 10 living in the village. RESULTS mean age were respectively: 29.4(5.3), 25.2(3.5), 10.8(1), 10.9(1); N 28.7(7), 25.8(5), 11.2(1), P 30.2(11), 23(4), 10.5 (2). Exhaled CO(ppm): 5.9(2), 4.1(2), 6(2), 7(2); N 8.3(3), 6.9(5.3), 21(4); P 7.3(2.4), 6.4(2.6), 5.2(1.4). SaO₂% 97.3, N 89.6, P 87.4. No difference was found among the groups in the age. SaO₂% decreases with altitude as expected; exhaled CO level is higher than expected in residents in rural village, especially in the two groups of women, despite no presence of traffic or other sources of environmental pollution. The use of biomass fuels and the lack of chimney is a common phenomenon in rural communities in the developing world and can affect respiratory health even in the absence of smoking habits, outdoor pollution and recurrent respiratory illness.

Supported by ZENECA

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HYPOXIA INCREASES VASCULAR PERMEABILITY IN HYPOXEMIC COPD PATIENTS.

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Prolonged exposure to hypoxia can increase vascular permeability. Actually, a microvascular leakage due to increased endothelial cell permeability has been described at high altitude. AIM: to investigate the effect of hypoxia on vascular permeability in hypoxic patients living at sea level. METHOD We examined the admission data (arterial blood gas, plasmatic and urinary proteins, an indirect index of increased glomerular permeability) of 58 COPD patients (17 females, 41 males) admitted to the hospital because of a deterioration of respiratory conditions (clinical and spirometric diagnosis). No one was on long term oxygen therapy. Patients affected by pneumonia (Chest X-ray diagnosis) or other infectious diseases, patients with elevated inflammatory indices, arterial hypertension, renal failure, diabetes, acidosis or other metabolic disturbances were excluded from the study. Patients were divided in 2 groups using $\text{PaO}_2=60\text{mmHg}$ as cut-off: H=hypoxic(28pt), N=normoxic(30). RESULTS mean (SD)

Age	PaO ₂ KPa	PaCO ₂ KPa	pH	Urinary Proteins
H	72.6(5) 6.8(0.2)	6.5(1.17)	7.39	18.3(40)g/dl ^{**}
N	76.8(2.9) 9.4(0.8)	5.9(0.6)	7.41	0.20(1)g/dl

A significant difference ($p<0.001$, paired t test) was found between H and N for PaO₂ and Urinary Proteins. A high correlation ($p<0.05$) was found between PaO₂ and Urinary Proteins in H patients. In H patients the vascular barrier to protein movement seems decreased and the transvascular protein escape increased. As we can reasonably exclude other causes of protein escape (i.e. renal failure, chronic infectious diseases, metabolic acidosis) hypoxia per se seems able to induce this effect on vascular permeability.

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EFFECT OF ACUTE, SEVERE HYPOXIA ON AIRWAY SMOOTH MUSCLE RESPONSIVENESS IN GUINEA PIGS.

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The hypoxia conditions can affect bronchial responsiveness (BR). Recently, we demonstrated a reduction of BR in asthmatics at high altitude (Eur Respir J 1995;8:1842-1846). AIM: to assess the mechanisms involved in this response we investigated whether in-vivo hypoxia affects BR in isolated guinea pig bronchi. METHODS: anaesthetized guinea pigs were exposed for 1 h, 2 h, or 3 h to 5% O₂ (in 5% CO₂, 90% N₂ =H) or air (21% O₂ =N), through a tracheal cannula. In-vitro bronchial smooth muscle responsiveness was after measured. Bronchial rings obtained from each guinea pig were kept in a organ bath with Krebs-Henseleit solution. In isometric conditions, we performed concentration-response curves to acetylcholine (ACh) and histamine (His) (10^{-2} to 10^{-6} M, n=11-16, mean \pm SEM). The EC₅₀ values were measured (n=11-16, mens GSEM, * $p<0.05$).

	1 h	2 h	3 h
ACh	2.8×10^{-5} (2.8)	1.9×10^{-5} (3.0)	2.7×10^{-5} (3.8)
N	1.8×10^{-5} (2.7)	1.3×10^{-5} (3.6)	2.8×10^{-5} (2.1)

	1 h	2 h	3 h
His	1.3×10^{-5} (0.3)*	1.2×10^{-5} (2.3)	3.3×10^{-5} (2.4)
N	7.7×10^{-5} (1.7)	1.6×10^{-5} (2.6)	1.6×10^{-5} (2.6)

RESULTS: we found a significantly higher EC₅₀ value for His curve, but not to ACh, in rings from H compared to N animals only at 1 h exposure, no difference was found at 2 and 3 h exposure. We found a significant reduction of the in-vitro BR to both ACh and His in H compared to N animals ($p<0.05$ by ANOVA) only at 3 h exposure, no differences were found at 1 and 2 h exposure. CONCLUSIONS: in-vivo 1h exposure to acute,severe hypoxia changes BR to His but not to ACh, 3 h exposure induces a significant reduction on BR to both ACh and His.

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BENZOLAMIDE, ACIDOSIS AND ACUTE MOUNTAIN SICKNESS.

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Benzolamide blocks carbonic anhydrase CAIV isoenzyme on the vascular endothelium and CA in the proximal tubule of the kidney. It does not cross the blood brain barrier and could be useful if it prevents acute mountain sickness (AMS). Twenty-two members of the BMEME were randomly allocated to receive either benzolamide (100mg b.d.) or matching placebo in a double-blind manner. They took active drug, or matching placebo, for 1 day before, and then during ascent to 5340m over a median of 12 days. Subjects completed a standardised AMS questionnaire. Benzolamide reduced total AMS scores, worst AMS scores (median 8 placebo, 3.5 benzolamide, $p<0.005$ C.I. 8.2 Mann-Whitney U test), and AMS scores on arrival at each new altitude ($p<0.003$, ranked medians ANOVA). Benzolamide treated subjects had higher oxygen saturations as they ascended ($p=0.005$ ANOVA). Benzolamide reduced AMS, and increased ventilation (reflected by reduced PaCO₂ and increased SaO₂), even with a normal ascent rate. Arterialised capillary blood gases obtained in the control group were consistent with the model of Wolff PaCO₂ expected = $0.25 \text{ PaCO}_2 + 70\text{pH} - 503 \text{ mmHg}$; expected PaCO₂ mean 27.2mmHg, observed mean 27.0mmHg, 95% C.I. of the difference -2.5,2.9 n.s.; paired t-test. Benzolamide treated subjects, however had PaCO₂ values significantly lower than the placebo group and incompatible with the model (expected mean PaCO₂ 26.1mmHg, observed mean 23.6mmHg, 95% C.I. of the difference 0.7, 4.5 $p=0.03$, paired t-test). Their results are incompatible with the model. This analysis of the study does not support metabolic acidosis as the mechanism explaining the drive to breathe on benzolamide. Blockade of CAIV, perhaps at the level of the blood-brain barrier, is suggested as a possible mechanism.

Wolff, C.B. *Molecular Aspects of Medicine* 1992 13 445-568.

ABSTRACTS

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POSTURAL SWAY DEFICIT AT HIGH ALTITUDE.
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Sensory deficits in the auditory system (cochlear sensitivity and sound localisation) have been shown on exposure to high altitude. In the present study we examined the influence of altitude on postural stability. Members of the BMEME were tested at four stages of the project; in the UK, on arrival at 5340m, after at least 7 days at 5340m, and after the expedition in the UK. Subjects stood on a Postural Sway Meter and sway was recorded for 30s for each of the three test conditions (a) eyes closed (b) eyes open (unfixated) (c) eyes open (fixated on an object about 2m away). Exposure to high altitude had no effect on postural sway when the eyes were closed 0.42±0.02deg (mean±SEM) at sea level compared with 0.41±0.02deg on arrival at 5340m. At sea level opening the eyes reduced postural sway to 0.30±0.01deg (n=73, unfixated) and 0.32±0.01deg (n=78 fixated). On arrival at base camp opening the eyes was still reduced sway, but more so when the subject fixated on an object. Thus the contribution of unfixated gaze on posture was reduced ($p<0.005$ test, n=73 sea level, n=52 @5340m). After acclimatisation to 5340m, the benefit of unfixated gaze on stability was restored. Our results indicate that the contributions of two types of visual input to the maintenance of posture are differently sensitive to altitude exposure. As the hypobaria, and experimental conditions did not change at 5340m, and this effect was abolished after acclimatisation, hypoxia is likely the mediator of the effect, arterial oxygen saturation increased from a mean of 78.6% (0.8 S.E.M.) on arrival at 5340m, to 82.4% (0.9) after acclimatisation ($p<0.005$ paired t-test). The site of this hypoxic effect is not shown by our study. The postural benefit from unfixated vision might be more sensitive to a deficit in attention or concentration. Unfixated gaze, like directional hearing, may be more sensitive to a deficit much higher in the postural control hierarchy, perhaps because of much increased processing required to use unfixated cues.

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SYSTEMIC BLOOD PRESSURE INCREASES DURING TREKKING ASCENT TO HIGH ALTITUDE IN MAN.

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There have been conflicting reports of elevation and reduction of systemic blood pressure during ascent to high altitude. Systemic blood pressure was measured using an aneroid sphygmomanometer (Accuson), in 41 members of the British Mount Everest Medical Expedition, during ascent to 5340m over an average of 12 days. Blood pressure in each subject was measured by the same observer throughout. The table shows the mean (SD) systolic and diastolic blood pressures (BP), and the pulse pressure, at different altitudes and the corresponding atmospheric pressure:

Atmospheric pressure (mBar)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Pulse pressure (mmHg)
850-900	115 (0.8)	72 (0.7)	43.1 (0.8)
701-750	117 (1.1)	78 (0.8)	39 (1.0)
651-700	119 (1.0)	78 (0.8)	41 (1.0)
601-650	120 (0.8)	80 (0.6)	40 (0.7)
551-600	124 (0.8)	82 (0.7)	42 (0.8)
501-550	128 (1.1)	83 (0.8)	44 (1.0)
450-500	129 (1.5)	83 (1.2)	46 (1.5)

Systolic blood pressure, diastolic blood pressure and pulse pressure increased during ascent ($p<0.001$, general linear model). This increase was apparent when sea-level data was excluded and within trek days at different altitudes were compared. Confounding variables, such as the effect of increasingly cold ambient conditions, cannot be excluded, but this study demonstrates a clear upward trend in blood pressure in a group of lowlanders on ascent to altitude

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GENDER AND WEIGHT LOSS AT HIGH ALTITUDE

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Weight loss is a common problem for climbers at high altitude. anecdotal reports suggest that women may be less prone to lose weight at altitude. We measured body mass using calibrated scales (Marsden Weighing, London) prior to postural stability tests at sea level in the UK, after arrival at 5340m, and after at least 7 days of sojourn at 5340m. Subjects' height had been measured in the UK. Body mass index (BMI weight/height²) was calculated. The change in BMI between arrival and post acclimatisation measurements (median time at or above 5340m: 15 days) was measured for 38 expedition members, and expressed as change in BMI/day, for 9 women and 29 men. Those men not exposed to altitudes over 7000m lost weight averaging 0.116 (0.026) kg/m²/day (95% C.I. 0.06-0.17, $p=0.0002$ test), compared with the 8 women, who did not lose a significant amount of weight, averaging 0.02 (0.03) kg/m²/day (95% C.I. -0.07-0.10, $p=0.01$ test). The difference between the changes in BMI was also significant ($p=0.03$ test). Whilst the 7 male high-altitude climbers exposed to altitudes between 7100m and 8848m all lost weight by an average of 0.15 kg/m²/day (range 0.11-0.21 kg/m²/day, 95% C.I. 0.11-0.19; $p=0.0003$ test) the one female high altitude climber (Alison Hargreaves) who spent 4 nights above 8000m without supplementary oxygen, retained her previous weight (57.4kg arrival b.c., 57.6kg after descent to b.c., her BMI increased by .004 kg/m²). All had been exposed to similar diet and environmental conditions, and differences in activity level do not explain the findings in either group. By whatever mechanism, women appear to maintain their weight at high altitude.

We would like to thank AJH's family for permission to relate her data.

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DOES TRAINING AND/OR RESIDENCE AT ALTITUDE IMPROVE SEA-LEVEL MAXIMAL AEROBIC POWER ($\dot{V}O_{max}$) AND ENDURANCE PERFORMANCE?

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There is no consensus as to whether altitude training and/or residence confer a benefit to the competitive athlete performing at sea level (SL). Twenty-six exercise studies were critically reviewed to determine whether there was sufficient basis to make comparisons and form a conclusion. Discounting training elevation and duration of exposure and initial fitness level, only 9 of 13 studies performed $\dot{V}O_{max}$ studies on return to sea level after training. Five of the nine reported 4-14% increases but lacked a SL control group. Four of the nine studies using a control group reported no improvement in $\dot{V}O_{max}$ either during or after altitude training. Only two of six other studies involving short-term hypoxic training and SL residence showed improvements in SL $\dot{V}O_{max}$ compared to a control group. Of seven additional studies involving training and residence at altitude, only two showed SL $\dot{V}O_{max}$ increases $>5%$, but four of the seven reported small increases in endurance performance. We conclude that: 1. all altitude/hypoxia training studies should have matched SL controls; and 2. despite the $\dot{V}O_{max}$ change, if any, resulting from the training, small individual changes may occur that would improve SL competitive endurance performance.

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RED BLOOD CELLS AND HYPOXIC PULMONARY VASOCONSTRICITION: HCT DEPENDENCE, AND THE ROLES OF NITRIC OXIDE AND ADENOSINE

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To explore the interaction between hypoxic pulmonary vasoconstriction (HPV) and red blood cells (RBCs), we studied mechanically ventilated, perfused lungs from euthanized New Zealand White rabbits. Lungs were perfused *in situ* in a recirculating manner using a pulsatile pump at constant flow; perfusates consisted of Krebs-Henseleit buffer, or buffer plus washed RBCs at a Hct of 10 or 30%. Repeated hypoxic challenges were administered over 120 minutes, and the hypoxic pressor response (HPR) was quantitated as the change in pulmonary artery pressure from baseline after 5 minutes of hypoxia. Expired and perfusate nitric oxide (NO) was measured using chemiluminescence, and perfusate adenosine levels were measured in a subset of experiments. HPR was greater at a higher Hct, and increased over time in the 30% Hct groups only. Expired NO varied inversely with Hct, and decreased with hypoxia and over time. Perfusate NO_x increased over time, but did not vary with hypoxia or Hct. Adenosine receptor blockade did not affect the Hct-dependence of HPV, and adenosine levels were virtually unmeasurable at any time during the experiments. We conclude that: 1. The strength of HPV is dependent on Hct; 2. Expired NO is inversely related to Hct; 3. Adenosine appears to play no role in the Hct-dependence of HPV; 4. A relationship between HPV, RBCs, and NO may exist.

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WOMEN AT ALTITUDE: THE EFFECT OF ACCLIMATIZATION ON BONE RESORPTION AS INDICATED BY DEOXYPYRIDINOLINE CROSSLINKS

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This study was designed to investigate how exposure to high altitude affects urinary markers of bone resorption in women. Sixteen women (mean age: 21.7 ± 0.5 years; mean ht: 167.4 ± 1.1 cm; mean wt: 62.2 ± 1.0 kg) were studied twice at sea level (SL) and once on Pikes Peak (PP) (4301 meters) for 12 days. Preliminary data are presented in this abstract on 5 of the 16 subjects. The subjects were on a controlled diet during each admission. Twenty-four hour urine collections were made for 5 days in each menstrual phase at sea level (SL) and 10 days in one phase on PP. Urine samples were analyzed for deoxypyridinoline crosslinks (a urinary marker of bone resorption) using a competitive enzyme immunoassay. Mean values at sea level were 4.3 ± 1.9 nM/mM creatinine, and at altitude were 5.9 ± 1.1 nM/mM creatinine.

Analysis of these 5 subjects suggests an increase in bone resorption at altitude relative to sea level.

This study was supported by the Department of Defense contract #DAMD-17-95-C-5110.

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 β -ENDORPHIN AFFECTS IMMUNE FUNCTION OF RATS DURING ACUTE HYPOXIA*

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To study the effects of β -endorphin on immune function during acute hypoxia. The lymphocyte proliferation and hemolysis formation were determined under iv β -endorphin or acute hypoxia with or without naltrexone. The results showed: Either acute hypoxia (7 km, 24 h) or iv β -endorphin (0.01 nmol 2 h) inhibited concanavalin A (Con A)-induced splenic T-cell proliferation and SRBC-sensitized hemolysis formation. Naltrexone, which per se did not show influence on T-cell proliferation and hemolysis formation, blocked partly the immunosuppressive effects of acute hypoxia. The results suggest that β -endorphin may modulate the immune response to hypoxia stress by opioid receptors.

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REDUCTION OF LUNG VOLUME AND EXPIRATORY FLOW RATE AT HIGH ALTITUDE: LONGITUDINAL MEASUREMENTS DURING AN EXPEDITION TO MUSTAGH ATA

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Introduction: International trekking tourism exposes an increasing number of people, some with pre-existing health problems, to high altitude. So far there are few data on the alterations of pulmonary function during a limited stay at high altitude. **Aim of the study:** Repeated assessments of expiratory flow rates and volumes (eFVC), oxygen saturation (SaO₂) and expiratory pCO₂ (etCO₂) at altitudes above 4000m to show whether adaptation to high altitude can be assessed by non-invasive measurements (SaO₂, etCO₂, eFVC). **Methods:** During an expedition to the Mustagh Ata mountain (7545m), all 15 healthy participants measured eFVC 3x/day with an electronic hand spirometer (AsthmaMonitor, Inc. Jaeger, Würzburg-Germany). In addition SaO₂, etCO₂, expiratory rate (RR), pulse rate (PR), altitude, temperature, air pressure and clinical symptoms (Acute Mountain Sickness Score) were recorded. **Results:** During ascents to altitudes above 4500m, a significant decrease of FVC, FEV₁, MEF25, MEF50 and MMEF (each $p < 0.001$, $n = 743$ measurements) was observed. Throughout the stay at high altitude those parameters were reduced by comparison with baseline measurements, and were normalized only after descent below 1500m. Changes in PEF were not significant. SaO₂, PR, RR and etCO₂ showed a significant inverse correlation to the altitude reached. One of the participants, who suffered high altitude pulmonary edema (HAPE), showed a marked reduction of FEV₁, FVC and MEF25 12 h prior to the onset of clinical symptoms, whereas SaO₂ was reduced only with the onset of HAPE.

Discussion: eFVC was significantly reduced during a stay at high altitude in poorly acclimatized healthy subjects. A more than usual decrease of eFVC could be an early indicator of impending HAPE. **Conclusion:** We would like to thank the participants, the DAV Summit Club and Inc. Jaeger, Germany, for their support.

ABSTRACTS

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INCIDENCE OF ACUTE MOUNTAIN SICKNESS IN A GENERAL TOURIST POPULATION IN THE ITALIAN ALPS

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Few data are available about the incidence of Acute Mountain Sickness (AMS) in a general population at moderate altitude. We studied two groups of people attending summer ski lessons in the Italian Alps: one stayed in the Stelvio area (S), between 2760 and 3100 m asl; the other was attending lessons in the same ski area, but the overnight stay was in Bormio (B), at 1200 m asl. Group B included 160 people, 107 M, 53 F, mean age 39.7 (R: 16-75), group S included 372 people, 254 M, 118 F, mean age 39.2 (R: 16-70). Subjects were evaluated with the Lake Louise AMS Scoring System. Diagnosis of AMS: headache and other symptoms, score ≥ 3 . Results: AMS cases in group B 11 (6.9 %), group S 47 (12.6 %), $p = 0.07$. Only in the S group some people reported moderate or severe activity reduction. In both groups AMS was significantly more frequent in females group B 8 F (15 %), 3 M (2.8 %) ($p < 0.05$), group S 26 F (22 %), 21 M (8.2 %) ($p < 0.001$). No cases were found in subjects aged ≤ 20 . Conclusions: in our study, AMS was significantly more frequent in females and rare in younger people. The incidence of AMS was lower than that reported in USA, but similar at the incidence described in European climbers.

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VENTRICULAR BLOCK FUNCTION IN HUMAN "NORMAL" HEART
(DATA FOR HYPOXIA STATE EVALUATION)

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From 1987 we published reports with performance of the heart as a whole organ analysis basing on anatomic and functional integral values. Anatomic integral values

Whole heart and its performance of the heart as a whole, we recognize three blocks in it located intrapericardially:

1 "atrial" block - left (LA) and right (RA) atrium;

2 "aorta-pulmonary" block - aorta bulb(A) and pulmonary artery(PA);

3 ventricular block - ventricle (V) including the pumping of left (LV) and right (RV) myocardial chambers, both with blood outflow into "aorta-pulmonary" block vessels, and

spongy (venous) myocardial chamber with the blood outline through coronary sinus (CS) and Thesleit Vein (TV) into "atrial" block.

Functional values (parameters) are partially:

The following concepts are introduced for Vb functions assessment:

A "overall" blood volume (as a sum of all three chambers volumes);

"overall" endovascular volume (overall EDV);

"overall" endo-venous volume ("overall" EEV);

1 "atrial" systolic and "ventricle" diastole of "three-chambered" Vb.

Heart pressure (Pi) findings (in mmHg) received in 18% persons, free from cardiovascular pathology according to catheterization data (practically "normal" heart). The data are presented.

The process of normal "ventricle" Vb systole:

- begins with blood ejection from Vb spongy chamber into the "atrial" block with "overall" EDV at the following Pi levels:

CS=12.0; PA=10.2; TV=7.7; LA=4.5; RA=4.5; LV=15.0; RV=15.0;

- contraction of blood outline from Vb into "aorta-pulmonary" block with venous minimum - x-collapses formation in "atrial" block equal to XRA=7.4±0.7; XLA=2.44±0.58;

- completed with the chamber collapse - Vb general emptying with "overall" EDV at the following Pi levels:

CS=1.5±0.3; PA=22.2±2.9; TV=25.1±1.27; LV=109.6±2.2; RV=107.4±2.6;

At this period the following blood volumes are transferring:

- two - from RV and LV (through aorta-pulmonary) into "aorta-pulmonary" block;

- two - from systemic and pulmonary veins into "atrial" block

during the process of so called "systolic" membrane suction (at pulling atrio-ventricular valves into LA and LV chambers) and blood ejection through these valves to the spongy Vb inflow "atrial" block by blood outline from "three-chambered" Vb into "aorta-pulmonary" block.

The preliminary reports are published in: 9th Intern. Hypoxia Symp., 1995.27. Intensive Care Medicine, 1995, v.28, suppl. 1, s141.

11th World Congress of Anesthesiologists, 1996, 294.

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SUMMABLE GAS PRESSURE (PO2+PCO2) TESTS IN THE EVALUATION OF
CEREBRAL GAS EXCHANGE IN HYPOTENSIVE HUMAN

(DATA FOR HYPOXIA STATE EVALUATION)

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Here we report the data on PO2,PCO2(mm Hg),SO2, pH tests that are widely used in cerebral gas exchange (SG) assessment, as well as the data on venous gas pressure (Vg) tests (PO2,PCO2 in mm Hg - in femoral arterial (x) and jugular venous (v) blood).

Tests of SG do not substitute conventional ones (PO2 and PCO2) but include them as a part, choosing only additive characteristic - pressure gradient (Pi-PO2) and gradient of SG. The gradients on SG have been calculated, gradient on SG was denoted as "gas functional" ($\Delta P_f=Pi-PO2$ at $Vg=V$) and also gradient ΔP_f "exchanged" equal to ΔP_f at $(PO2_f-PO2_f)$ at $Vg=V$.

The mean pressures (in mm Hg) in the following sites: arterial sigmoid sinus (Pi), jugular veins (Pi), jugular veins (PO2), jugular veins (PCO2), arterial (a) and venous (v) blood, arterial (Pi), hemoglobin (Hb) and hematocrit (Ht) have been evaluated for the characteristics of background condition at the CCE investigation. The data were derived from 86 persons free of cardiovascular pathology according to their catheterization data, during spontaneous air breathing, in supine position and at rest (so-called "practically normal").

PO2 PO2_f Pi PE pH SO2
Ar 95.7±0.6; 97.3±2.3; 123.1±8.47 94.2±0.16 7.39±0.004
V 93.7±0.5; 94.8±2.24 84.8±0.54 44.2±0.16 7.35±0.004
 ΔP_f 47.7±0.6; 9.55±0.26 38.22±0.54 28.47±0.74 0.03±0.002
 ΔP_f 47.7±0.6; 2.78±0.22 11.12±0.14 8t=45.0±1.2
Hb=13.1±0.5; Ht=41.2±1.2

It is worth to note that the arterial blood pressure examination (supine position, air breathing) and the attended values, summary gas pressure in venous blood (outflowing from the brain (v)) is close to $PO2$ in arterial blood flowing to the brain.

Gradients of ΔP_f at $Vg=V$ are 8.0±0.57 mm Hg. It appears to be a measure of adequacy for making unidirectional shifts of PO2 in ar blood and SG in Vg blood. It could be positive, zero, negative or change both on magnitude and in sign, but it didn't reach while "normal" the absolute value of ΔP_f "exchanged" positive, negative or zero. In arterial hypoxia, ΔP_f "exchanged" (negative) for kidneys (-11.9±0.57 mm Hg). All the published before characteristics of SG tests are extended to the gradient ΔP_f "exchanged" (1-6). We believe that the data presented can be used for SG tests in arterial hypoxia, as well as in arterial hypoxia.

References: 1. Intern. Soc. Pathophysiol. Moscow, 1991, 318; 2. Intern. Hypoxia Symp., 1995, 20, Suppl. 2, 540, S140; 3. 9th Europ. Congr. Anesthes., Jerusalem, 1994, 210, 474, 513, 514; 4. 5th Intern. Hypoxia Symp., 1995, 27; 5. Cardiovascular Research, Sept. 1995, 31, 1; 6. Intern. Care Medicine, 1995, v.28, suppl. 1, s141; 7. 11th World Congress of Anesthesiologists, 1996, 294.

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SUMMARY GAS PRESSURE TESTS(PO2+PCO2) IN "NORMAL" HUMAN MYOCARDIAL
GAS EXCHANGE EVALUATION (DATA FOR HYPOXIA STATE DIAGNOSIS)

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Heart gas exchange values are used for myocardial state evaluation. Our first report on the subject was published in 1962 [F. Fedon, Kapane works, 1962, N.4, p. 18-26].

Here we present the data on PO2,PCO2 (mm Hg),SO2,pH, as well as on summary gas pressure(SGP),calculated from $PO2/(PO2+PCO2)$ in both femoral artery (a) and s.coronaria (sc) blood. Arterio-venous gradients have been calculated by these values ($\Delta(a-sc)$). Gradient on SG was denoted as "gas functional" ($\Delta P_f=Pi-PO2$); Gradient ΔP_f "exchanged", that is equal to $PO2_f/(PO2_f+PCO2_f)$ have been also calculated. These data were derived from 186 subjects free of cardiovascular pathology according to catheterization data during their spontaneous air breathing ("normal"). There were cases with PCO2 in ar from 30 to 45 mm Hg and PO2 in ar from 70 to 100 mm Hg.

	PO2	PO2_f	PE	pH	SO2
a	91.7±0.5	36.6±0.2	121.1±2.0	7.39±0.003	96.7±2.8
sc	23.7±0.3	47.5±0.2	7.32±0.6	7.34±0.005	38.20±0.7
(a-sc)	67.9±0.4	10.0±0.2	51.8±0.4	0.057±0.002	56.7±0.7

The data presented showed that in "normal" humans:

- PO2 level in sc blood is less than PO2 level in sc blood;

- PO2,PO2_f and PE levels in sc are the least out of all the vascular bed sites (bulbus v.jugularis, v.hepatice,v.renalis, a.pulmonalis), the data on these levels were published earlier [1-7];

- gradients on PO2,SO2 and "gas functional" are maximum;

- ΔP_f "exchanged" value for the heart is positive (26.6±0.44 mm Hg), contrary to ΔP_f "exchanged" one for kidneys, having negative value (-11.9±0.57 mm Hg).

The preliminary reports are published in:

1. Intern. Soc. Pathophysiol. Moscow, 1991, 318; 2. Intern. Care Medicine, 1995, 20, Suppl. 2, 540, S140; 3. 9th Europ. Congr. Anesthes., Jerusalem, 1994, 210, 474, 513, 514; 4. 5th Intern. Hypoxia Symp., 1995, 27; 5. Cardiovascular Research, Sept. 1995, 31, 1; 6. Intern. Care Medicine, 1995, v.28, suppl. 1, s141; 7. 11th World Congress of Anesthesiologists, 1996, 294.

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NEUROPATHOLOGICAL CHANGES IN NEONATAL DOGS FOLLOWING BILATERAL CAROTID ARTERY OCCLUSION -A DOG MODEL OF HYPOXIC-ISCHEMIC ENCEPHALOPATHY-
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Hypoxic-ischemic encephalopathy (HIE) is brain injury found in infants, and is the most common lesion responsible for cerebral palsy. Here we report chronic neuropathological changes in neonatal dogs after bilateral carotid artery occlusion (BCAO). At 14 days of age, seven mongrel puppies were anesthetized and bilateral carotid arteries were ligated with sutures. Seven sham-operated littermates served as controls. They were sacrificed at three months of age, and their brains were examined macro- and microscopically. Neuropathological examination revealed dilated posterior communicating and basilar arteries in BCAO group. Six out of 7 BCAO brains had uni- or multiloculated cysts surrounded by a band of GFAP-positive glial cells. Scattered small areas of gliosis in the periventricular white matter. Four showed ventricular dilatation. Although the cerebral cortex seemed to be intact, the periventricular white matter and corpus callosum were reduced in width. Myelination in the white matter was significantly reduced in BCAO animals compared with the controls. These neuropathological changes were similar with those in periventricular leukomalacia. Periventricular leukomalacia is one of neuropathological varieties of neonatal HIE and is usually found in preterm human infants.

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THE EFFECTS OF VOLUNTARY HYPOVENTILATION IN WOMEN IN MOUNTAINS
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The ordinary regulation of respiration doesn't ensure the effective adaptation to hypoxia. The information about the possibility of use the voluntary respiration with this purpose in women is absent. Our aim was to study cardiorespiratory (CR) responses to voluntary hypoventilation (VH) in women during the adaptation to mountains. We examined 5 healthy women 28-49 years old in normal conditions, on 1 and 5 days in the mountains of Caucasus (2100m) at rest, during and after hypoventilation test (1 respiratory cycle in a minute for 20 min.). This regime of VH required preliminary everyday breathing training for 3 months. The parameters of external respiration and interchange of gases, ECG were registered. The results showed that CR responses to VH in normal and hypoxic conditions had the same dynamics. But in the mountains the changes of the respiratory parameters were more distinct. The use of breathing training in normal conditions led to the formation of the new stereotype of respiration, which remained in the mountains. So we concluded that it is possible to make a prognosis of the effects of VH in women in mountains, using this breathing test on earth,

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ARTERIAL AND VENOUS OXYHAEBOGLOBIN SATURATION (S_o_2 AND S_vo_2) DURING FEEDING IN THE YOUNG LAMB.
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Cyanosis during feeding is a common clinical complication in the newborn. Because the rate of arterial desaturation is strongly related to the pre-desaturation level of S_o_2 during repeated apneas (Wilkinson et al., Resp. Physiol., 101:321-331, 1995) we sought to determine if S_o_2 remains decreased throughout the feeding period, thus creating a condition favouring rapid arterial desaturation. One epoch of feeding was recorded from 5 lambs (19-27 days old) instrumented to record cardiac output (flow probe), S_o_2 and S_vo_2 (fiberoptic catheter oximeters). On average, an feeding epoch was composed of 7 ± 2 (mean ± SEM) periods of sucking (duration 1.2 ± 4 sec) separated by periods of non-sucking (duration 6 ± 2 sec). During a feeding epoch, arterial blood sequentially desaturated and re-saturated during the periods of sucking and non-sucking respectively. The nadir of these arterial desaturations (83% ± 2%) were significantly lower than control values (92% ± 2%, P<0.05, ANOVA). During periods of non-sucking, S_o_2 returned to the control level. In contrast, S_o_2 decreased and remained significantly lower than control levels (55% ± 3%) throughout the feeding epoch (46% ± 3%, P<0.05). Total body oxygen consumption was significantly increased from control levels during the non-sucking periods (10.9 ± 1.1 mlO₂/min/kg control, 13.9 ± 1.2 mlO₂/min/kg non-sucking, P<0.05). This increase in oxygen consumption was provided for by a significant increase in total body oxygen extraction as systemic oxygen transport was unchanged. In conclusion, our results show that during feeding, body stores of oxygen (S_o_2) decrease and remain decreased throughout the feeding period. A reduced S_o_2 , coupled with elevated oxygen consumption, may be the predisposing factors that lead to rapid arterial desaturation and cyanosis during feeding.

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ACUTE SIMULATED ALTITUDE ATTENUATES POSTPRANDIAL HUMAN SUPERIOR MESENTERIC ARTERY BLOOD FLOW
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Reduced blood flow to the gut may contribute to weight loss and GI symptoms at altitude. Previously reported uncontrolled field studies with chronic hypoxia (>48 hrs) at Mt. McKinley demonstrated a significant reduction in postprandial superior mesenteric blood flow. Accordingly, we tested the hypothesis that controlled, acute simulated altitude attenuates normal postprandial hyperemia in the human superior mesenteric artery. Using previously validated noninvasive ultrasonic Doppler flowmetry, we measured phasic superior mesenteric artery blood flow (MQ) and cardiac output (CO) in 9 (3 females) healthy adults (age 24±6 yrs, BSA, 1.77±0.1 m²). Data was obtained in a hypobaric chamber after 2 hours at 430 Torr, and the next day at 640 Torr. All measurements were made after a 12 hour fasting (PRE) and approximately 45 minutes after ingesting a 1000 kcal, 1 liter liquid meal (POST). Heart rate (HR) and mean cuff blood pressure (BP) were also measured. (* p<0.05 vs PRE; X±SD)

	640 TORR		430 TORR	
	PRE	POST	PRE	POST
HR(bpm)	58±9	66±10	71±13	80±14
BP(mmHG)	83±8	83±8	89±7	86±9
CO(L/min)	5.18±0.93	5.85±1.51	6.02±0.77	6.49±0.91
MQ(ml/min)	574±107	1235±269*	486±101	849±227*

Seven subjects had a mean LL/AMS symptom score of 3. Postprandial MQ increased 116±38% at 640 Torr versus 73±28% at 430 Torr (p<0.05). Results suggest that simulated altitude significantly attenuates postprandial hyperemia in the human superior mesenteric artery.

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ALVEOLAR HEMORRHAGE AS A COMPONENT OF HIGH ALTITUDE PULMONARY EDEMA (HAPE)

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We present the case of a 28 year old male climber who developed HAPE during an ascent of Mt. McKinley, Alaska. Analysis of lung lavage fluid showed an increased permeability pulmonary edema and alveolar hemorrhage. The subject was previously healthy and without significant prior illnesses and denied a prior history of HAPE. After a 3 day ascent from 2100 to 4200m he presented with dyspnea at rest and cough productive of pink frothy sputum. He had a pulse of 111 bpm, respiratory rate of 20/min, an SaO₂ of 74%, central cyanosis, and bilateral inspiratory crackles on chest auscultation. Fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) was performed in the lingula with return of serosanguinous fluid. After treatment with oxygen overnight he descended the next day and experienced complete resolution of symptoms. Analysis of BAL fluid at the 4200m camp showed a total cell count of 226,000 cells/ml, (altitude controls 79,000±15,000 cells/ml, n=5), and total protein of 258 mg/dl (altitude controls 5.8±3 mg/dl). Differential was 94% macrophages, 4% neutrophils and 3% lymphocytes. Remarkable were the numerous red blood cells (4,520,000 cells/ml) and foamy hemosiderin-laden macrophages, consistent with alveolar hemorrhage (BAL hemosiderin score of 139). This suggests that alveolar hemorrhage was present for at least 48 hours prior to the subject's presentation at 4200m, and during ascent before onset of symptoms. Given the known association of HAPE with increased pulmonary artery pressure, we suggest that alveolar hemorrhage in HAPE results from increased pressure and pulmonary capillary microvascular rupture resulting in inflammation and increased permeability edema.

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BIOCHEMICAL CORRELATES OF ADAPTATION TO INTERVAL HYPOXIA IN HEALTHY MEN

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Interval hypoxic training (IHT) is widely used in medicine for treatment and prophylaxis of different diseases. The aim of this study was to reveal changes of most important biochemical indices in blood of young healthy volunteers in the course of IHT and to search for criteria of adaptation to interval hypoxia. 18 male volunteers aged 18-20 years were subjected to hypoxic test (HT, inhalation of hypoxic gas mixture containing 11% O₂ during 10 min) and subsequent IHT (10-15 sessions of interval hypoxia, 1 session/day). HT induced decrease of uric acid, glucose and ethanol, increase of puruvate content in blood serum as well as changes of hormonal status. IHT abolished most changes seen after HT. The level of cortisol decreased and bilirubin level increased after 10 IHT sessions, both indices being within the normal range after 15 sessions of IHT. Patterns of cross-correlations between different metabolites and hormones were quite different before HT, after HT, and after IHT. Correlations including prolactin and luteotropic hormone were most abundant in different situations. Canonical correlations between sets of metabolites (glucose, lactate, urea acid, creatinine) and hormones (prolactin, luteotropic hormone, testosterone, thyroxine and cortisol) were evident before HT, disappeared after HT and re-appeared after IHT. The results suggest that normalization of hormone and metabolic levels, their "supercompensative" changes, restoration of hormonal-metabolic homeostasis regulation, as well as the appearance of new relations reflecting optimization of regulatory processes may serve as criteria of adaptation to IHT.

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ASSOCIATION OF HIGH ALTITUDE PULMONARY EDEMA WITH THE MAJOR HISTOCOMPATIBILITY COMPLEX.

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To examine whether constitutional susceptibility is present in high altitude pulmonary edema (HAPE)-susceptible subjects, we performed human leukocyte antigen (HLA) typing. The frequencies of HLA alleles in 28 male and 2 female subjects with a history of HAPE were compared with those in 100 healthy volunteers. The pulmonary hemodynamics on admission were retrospectively examined in 10 of the HAPE-susceptible subjects. The frequencies of HLA DR6 and DQ4 were significantly higher the subjects with HAPE than in the control subjects. DR6 was positive in 14 (46.7%) of the subjects with HAPE, but only 16.0% of the control subjects ($p = 0.0005$), and DQ4 was positive in 12 (40.0%) of the subjects with HAPE, but only 10.0% of the control subjects ($p = 0.0001$). The pulmonary arterial pressure on admission of the DR6-positive subjects with HAPE was significantly higher than that of the DR6-negative subjects with HAPE.

This study revealed a significant association of HAPE with HLA DR6 and DQ4, and of HLA DR6 with pulmonary hypertension. We conclude that HAPE, at least in some of its forms, reflects a host response to the environment at high altitude determined by HLA class II alleles located within the major histocompatibility complex.

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FELODIPINE TREATMENT AT HIGH ALTITUDE

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Background Felodipine is a dihydropyridine-type calcium antagonist with high selectivity for resistance vessels. It is widely used in the treatment of hypertension. However, it is not known whether felodipine treatment at high altitude will reduce blood pressure in healthy subjects. In the present study we wanted to assess the effects of felodipine SR on blood pressure and cardiovascular responses to cold pressor test at high altitude. **Material and methods** One female and 15 male healthy climbers participated. They were all members of a climbing expedition to Mt. Everest. All were examined at an altitude of 5200 m after four weeks of acclimatization. Felodipine was tested by a randomized, double-blind, placebo controlled, crossover design. Placebo or Felodipine SR 5 mg od were administered for two days. There was a wash-out period of three days before cross-over. The subjects were examined 3 to 6 hours after oral administration on the second day of treatment. Heart rate, BP and oxygen saturation were registered after 10 min sitting, 1 min cold pressor test and 10 min recovery. **Results** Felodipine SR did not change resting BP, heart rate or oxygen saturation. In all subjects blood pressure increased significantly during the cold pressor test, but there were no differences between placebo and felodipine SR. In conclusion, two days of felodipine treatment did not change blood pressure or cardiovascular stress responses.

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REGULATION OF GLYCOGEN PHOSPHORYLASE AND PYRUVATE DEHYDROGENASE AT DIFFERENT EXERCISE POWER OUTPUTS

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This study investigated the transformation and post-transformational control of glycogen phosphorylase (PHOS) and pyruvate dehydrogenase (PDH) at three exercise power outputs (35, 65 and 90 % of $\dot{V}O_{2\text{max}}$). Seven untrained subjects cycled at one power output for 10 min on three separate occasions, with muscle biopsies taken at rest, and 1 and 10 min of exercise. Glycogen phosphorylase in the active form (PHOSa) was not different across power outputs (21.4 - 29.6%), with the exception of 90%, where PHOSa fell significantly at 10 min (15.3%). PDH activation increased significantly from rest as a function of power output and time. Muscle lactate, acetyl-CoA, acetyl carnitine, free ADP, free AMP, and free P_i were unchanged at 35% $\dot{V}O_{2\text{max}}$, but rose significantly at 65% and 90%, with accumulations at 90% being higher than 65%. Glucose and G-6-P increased only at 90% and citrate was generally unchanged. Muscle glycogenolysis at 90% was 124.4 ± 14.0 mmol/kg dry mass in 10 min. In summary, PHOS activation was unchanged with increasing power outputs, despite the required increase in glycogenolytic flux. This suggests that the rate of glycogenolysis is regulated by post-transformational factor(s), such as the increases in the substrate, free P_i and the allosteric modulator, free AMP. Despite the increases in acetyl-CoA, PDH activation rose with increased power output, mirroring the need for increased CHO oxidation.

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ACCENTUATED HYPOXEMIA PRECEDES IMPAIRMENT OF GAS EXCHANGE IN SUBJECTS WITH AMS

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To closely investigate the early time course of changes in ventilation and gas exchange in the development of acute mountain sickness (AMS) 18 subjects were examined after passive ascent from 520 m (low altitude, LA) to 4559 m (high altitude, HA) under conditions of controlled fluid and sodium intake. Resting ventilation, petCO_2 , petO_2 as well as capillary blood gas analysis were obtained and alveolar-arterial O₂-difference (AaDO₂) calculated for LA and hour 1, 5, 10, 22 at HA. Additionally resting oxygen saturation (O_{2sat}) was recorded by pulse oximeter. 10 subjects were considered to have AMS (AMS+) because their functional Lake Louise score exceeded 1 (controls n=8). For main findings see table (mean \pm SD) * for p<0.05

	time at HA	1 h	5 h	10 h	22 h
O_2sat	AMS+	73.9 \pm 6.9*	76.1 \pm 6.5	77.6 \pm 4.0*	75.7 \pm 7.7*
	controls	80.0 \pm 4.0	79.3 \pm 6.5	82.5 \pm 5.3	82.8 \pm 3.7
petCO_2	AMS+	33.7 \pm 4.1*	31.0 \pm 4.2	30.3 \pm 2.1*	29.3 \pm 2.3
	controls	30.1 \pm 2.3	29.2 \pm 2.9	27.8 \pm 2.4	27.5 \pm 2.3
AaDO_2	AMS+	8.1 \pm 2.0	9.0 \pm 3.4	11.2 \pm 3.6	8.9 \pm 1.9*
	controls	6.5 \pm 1.4	9.7 \pm 3.2	8.4 \pm 2.9	6.3 \pm 1.5

We conclude that subjects developing AMS initially reveal accentuated hypoxemia due to alveolar hypoventilation which after 22 h is aggravated by impaired gas exchange.

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ANALYSIS OF THE EARLY HYPOXIC DIURETIC RESPONSE: EFFECTS OF HYPOXEMIA, HYPOCAPNIA AND HYPERPNEA AND THE PREDICTION BY CHEMOSENSITIVITY.

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Objective: The present study intended to evaluate the possible contribution of hypoxemia, hypocapnia and hyperpnea to the early hypoxic diuretic response (HDR) in humans. This approach was chosen to study the significance of the hypoxic ventilatory response in predicting the HDR. **Methods:** 13 male healthy subjects (aged 19-38) were examined at 4 consecutive days at the following conditions lasting 90 min each: 1) poikilocapnic hypoxia, 2) isocapnic hypoxia 3) normoxic control (random order, double blind). On the 4th day, voluntary hyperpnea was studied at a level corresponding to 2). O₂-saturation at 1) and 2) was kept around the level reached after 30 min of breathing 12%O₂ (i.e. after ventilatory depression) in a pretest. Sodium intake was controlled at 120 mmol/day to obtain an equilibrium within 5 days before and during tests 1)-4). Test conditions were identical between tests. **Measurements:** Urine samples were obtained for volume and sodium. Ventilation, FiO_2 , petO_2 and petCO_2 were analysed breath-by-breath. Oxygen saturation was recorded continuously. The hypoxic ventilatory responses (HVR) were measured under isocapnic (HVRiso) and poikilocapnic (HVRpoik) conditions beforehand. **Results:** 1) poikilo- and 2) isocapnic hypoxia and 4) hyperpnea led to an increase of diuresis of 1) 298.6%, 2) 211.1%, and 4) 180.1% of pretest samples. Significant differences to 3) normoxia were present for 1) and 2) only. Urine sodium revealed no such differences. Neither these hypoxic increases of diuresis nor the hypoxic sodium excretion correlated to HVRiso or HVRpoik. **Discussion:** As a main finding the early HDR does not involve natriuresis. Hyperpnea and hypocapnia appear to be minor contributors to the HDR. A simple relationship of HVRiso or HVRpoik to the early HDR or hypoxic sodium excretion is absent.

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DIFFERENTIATION OF FORWARD- AND BACKWARD WAVES IN THE PULMONARY ARTERY USING WAVE-INTENSITY ANALYSIS

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Pressure (P) and velocity (U) waveforms are the sum of incident and reflected waves. Traditionally, the calculation of impedance and phase angles (frequency domain) have been used to evaluate wave reflections. Wave-intensity analysis (WIA) is a new method of using high-fidelity P and U measurements to determine the direction and type of waves that are present at any given moment (time domain). Forward- and backward-travelling waves can be characterized as compressive (like blowing) or expansive (like sucking). In 6 open-chest anaesthetized dogs, we inserted a counter-pulsation balloon in the left atrium (LA) (25 ± 3 mL He) to introduce brief, single, pressure pulses. We measured pressure (Millar) in the LA, left ventricle (LV), RV, and PA, as well as U_{PA} (ultrasonic flowmeter). Pulmonary wedge pressure (PWP) was measured with a time-corrected fluid-filled catheter. LVEDP was set to ≥ 15 mm Hg. P_{PA} and U_{PA} were used for the WIA. Early during ventricular systole, balloon inflations increased P_{LA} by 14.2 ± 1.9 (SEM) mm Hg. Simultaneously, P_{LV} , P_{RV} , and P_{PA} increased by 4.1 ± 1.1 , 6.0 ± 0.6 , and 4.8 ± 0.6 mm Hg, respectively. U_{PA} increased from 89.1 ± 8.8 to 109.0 ± 9.4 cm/s ($p < 0.0001$) resulting in an augmented forward compression wave (by 11.3 ± 3.0 W/m²). Because the PWP wave arrived 79 ± 9 ms after the rise in P_{PA} , thus these changes could not have gone through the pulmonary circulation but through the heart. The arrival of the backward wave transmitted through the pulmonary circulation was seen as an increase in PWP (6.9 ± 1.5 mm Hg), causing a second rise in P_{PA} then P_{RV} , causing a backward compression wave (4.1 ± 0.8 W/m²). We conclude that WIA can be used to accurately quantify the direction and character of waves as they appear in the PA.

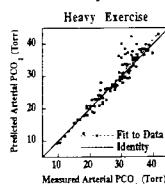
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NO EVIDENCE FOR PULMONARY DIFFUSION LIMITATION OF CARBON DIOXIDE EXCHANGE DURING EXERCISE AT SEA LEVEL AND EXTREME ALTITUDE

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Although CO_2 is 20 times more soluble than O_2 in the blood-gas barrier, because of the steep slope of the CO_2 dissociation curve and the time required for chemical reactions of CO_2 in the blood, the calculated equilibration time in the pulmonary capillary is similar for CO_2 and O_2 . Pulmonary diffusion limitation of O_2 during exercise is well established, both at sea level in athletes capable of very heavy aerobic work, and at altitude, when the slope of the O_2 dissociation curve increases. We used previously published multiple inert gas elimination (MIGET) data to test the hypothesis that pulmonary diffusion limitation of CO_2 occurs under the same experimental conditions that produce diffusion limitation of O_2 . Pictured is the relationship between measured PaCO_2 and PaCO_2 predicted from the MIGET for subjects ($n=50$) exercising at sea level ($\text{VO}_2 = 2.2-5.1 \text{ L/min}$) and simulated altitude ($\text{Pbar} = 523-240 \text{ Torr}$). In contrast to the PaO_2 data in the same subjects, there is no systematic difference between measured and predicted values of PaCO_2 . Thus there is no evidence for diffusion limitation of CO_2 under any condition, even when there is pulmonary diffusion limitation of O_2 . We suggest that the speed of the chemical reactions for CO_2 determined in vitro may underestimate the *in vivo* values.



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PULMONARY BLOOD FLOW, LUNG DIFFUSION AND HCVR IN AN EARLY PHASE OF A SIMULATED MICROGRAVITY

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Serial change of cardiopulmonary responses and hypercapnic ventilatory response (HCVR) to an early phase (5', 30', 1h, 3h, 6h) of a simulated microgravity, i.e. 6° head-down tilt bedrest (HDT), were investigated in ten male volunteers. Beat to beat change of stroke volume (SV) and cardiac output (CO) were measured by an impedance cardiography. Effective pulmonary blood flow (Qe), diffusion capacity of carbon monoxide (DLco) and functional residual capacity (FRC) were measured by multi gas rebreathing technique using a mass spectrometer. Minute ventilation (VE) and metabolisms as well as respiratory timings were monitored by a rapid response gas analyzer. Arterial blood gas analysis was done in each phase. HCVR was assessed by Read's method. 1. After an initial increase (from sitting to supine), SV and CO gradually decreased (-12% and -16% respectively). In contrast, Qe increased by 20% after 6h HDT. 2. DLco were preserved during HDT. Except marked initial decrease (-35%), FRC remained constant during HDT, then DLco/Ve showed a similar profile as DLco. 3. No significant changes in VE, VO_2 , VCO_2 , PaCO_2 and HCVR were found. 4. PaO_2 was decreased and AaDO_2 was enhanced after 6h HDT. It was suggested that, despite steady increase in pulmonary blood flow and DLco/Ve, pulmonary gas exchange became impaired because of relative reduction in Dm and remaining V/Q inequality which might be independent of gravity in an early phase of a simulated microgravity.

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THE EFFECTS OF PHYSICAL EXERCISE AND HIGH ALTITUDE EXPOSURE ON MUSCLE PROTEIN SYNTHESIS

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Prolonged high altitude exposure leads to considerable weight loss, in particular a reduction in muscle mass. In a chamber experiment, acute hypoxia resulted in a net loss of amino acids from forearm muscles, possibly due to a fall in muscle protein synthesis. However, physical exercise might be an important confounding factor counterbalancing the postulated decrease in muscle protein synthesis. Furthermore, an increase in extracellular pH was shown to stimulate protein synthesis. Therefore, we have investigated the effects of high altitude exposure with or without physical exercise on muscle protein synthesis in healthy volunteers. We measured muscle protein synthesis rates by the flooding technique before (at 550 m) and after passive (by helicopter; AIR-group; n=8) or active (by walking; FOOT-group; n=7) ascent to 4559 m (Cappanna Margherita, Swiss-Italian Alps) in healthy volunteers. [²H]arginylphenylalanine (3 g per 70 kg body weight, 10 and 20 atom-%) was iv. injected over 10 min, and blood samples drawn at intervals up to 90 min. Needle muscle biopsies were taken from the right vastus lateralis muscle at 90 min and immediately frozen in liquid N₂. The enchainments of [²H]arginylphenylalanine in the plasma free phenylalanine and in the muscle protein were measured by gas chromatography-mass spectrometry. In the AIR-group, the fractional rate of muscle protein synthesis was 2.16±0.35 %/d (mean±SD) at 550 m and 2.21±0.25 %/d at high altitude ($p=0.77$ by paired *t*-test). In contrast, the FOOT-group exhibited a significant increase in muscle protein synthesis from 1.82±0.35 %/d to 2.46±0.46 %/d ($p<0.001$). Also, the mean oxygen tension and the degree of respiratory alkalosis (assessed by arterial blood gas analyses) were not statistically different between the 2 groups at high altitude. In conclusion, hypobaric hypoxia had no influence on muscle protein synthesis. However, these data suggest that, in the short-term, physical exercise up-regulated muscle protein synthesis, irrespective of the degree of respiratory alkalosis.

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ACETAZOLAMIDE DOES NOT INCREASE CEREBRAL BLOOD FLOW VELOCITY AT HIGH ALTITUDE

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The effect on the cerebral blood flow of Acetazolamide (Az) as a prophylactic for acute mountain sickness (AMS) has not been evaluated at high altitude. In this study, pulse oxygen saturation (SpO_2), transcutaneous pCO_2 (pCO_2) and blood flow velocity of the middle cerebral artery (Vmca) using transcranial Doppler, was measured in two groups of volunteers at 4300 m. One group had prophylactic Az, 125-250 mg twice daily for at least 2 days (Az+). A second group had no Az (Az-). All subjects were at least 4 days above 2800 m, of which 3 days above 3440 m, and had no AMS, having 0-2 points on the Lake Louise self-report questionnaire.

Results: Values are mean ± SD. * $P = 0.0002$.

Groups	n	age (yrs)	sex (male, %)	pCO_2 (mmHg)	SpO_2 (%)	Vmca (cm/s)
Az-	39	34 ± 11	30 (77)	30 ± 5	86 ± 3	55 ± 14
Az+	12	30 ± 6	8 (67)	24 ± 4*	88 ± 4	60 ± 13

This study shows that Vmca and SpO_2 in the Az+ group are not different from those in the Az- group, but that pCO_2 is significantly lower in the Az+ group.

These data support the hypothesis that prophylactic use of Az has no effect on cerebral blood flow, and may not compromise intracranial compliance. The main effect of Az treatment is an increase of ventilation.

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IS CYTOPROTECTION BY FRUCTOSE 1,6-BISPHOSPHATE DURING HYPOXIA MEDIATED BY AN EXTRACELLULAR MECHANISM?

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Fructose 1,6-bisphosphate (F16BP) has been shown to protect organs from hypoxic and ischemic injury, presumably by potentiating the cellular energy state. Since it is unclear whether the highly charged F16BP molecule can cross the cellular membrane, we examined whether F16BP may be exerting its cytoprotective effect by an extracellular mechanism. Studies were performed on WI-38 cells, a liver derived cell line intolerant of anoxia. Hypoxic conditions were simulated by addition of antimycin A to the extracellular medium and cell viability was assessed by LDH release and energy charge. The continuous presence of F16BP did not significantly reduce LDH release, but significantly reduced LDH release after 3 hr (Table 1) (Miller *et al.*, ASCA, 1096). F16BP maintained energy charge significantly above control. A 10 min exposure to F16BP followed by removal reduced LDH release during subsequent antimycin treatment.

Treatment	Concentration mM	% LDH release
no antimycin	-	0
no compound	-	100
fructose	30	17
fructose 1,5-bisphosphate	30	37
fructose 2,6-bisphosphate	20	66
fructose 1,6-bisphosphate	30	67
glucose 1,6-bisphosphate	30	87
fructose 1-phosphate	30	100
fructose 6-phosphate	30	100

The cytoprotection afforded by these novel sugar bisphosphates, some of which are unlikely to participate in glycolysis, suggests a mechanism of cytoprotection at least partially independent of glycolysis. The continuous presence of F16BP is not required for protection, is consistent with an extracellular mechanism of action.

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EFFECT OF ACUTE HYPOXIA ON ARTERIAL AND VENOUS CATECHOLAMINE RESPONSES IN HUMANS

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Ten males and eight females (18-35 yrs) breathed 12% O₂ (4300M) for 8 hrs in a seated position. Arterial (A) and venous (V) blood samples were collected from indwelling catheters. ESQ and Lake Louise scores (LLS) were collected every two hrs. Plasma Epinephrine (EPI) and norepinephrine (NE) levels were determined by HPLC. Four males and four females demonstrated symptoms of acute mountain sickness (AMS+) as documented by ESQ and LLS. AMS+ had significantly ($p<0.05$) higher A-EPI levels at 45 and 240 min of exposure as compared to non sick subjects (AMS-). AMS+ also had higher V-EPI responses than AMS- but this was not reach significance. AMS+ had significantly higher A-NE at rest and for the first 60 min of hypoxia compared to AMS-. Control levels of A-NE and V-NE were significantly higher in AMS+. There was a significant correlation between AMS+ and high basal A-NE and V-NE. These findings suggest that individuals with high basal levels of A-NE and V-NE are more susceptible to hypoxia induced AMS. Further studies involving hypobaric hypoxia are indicated.

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THE ROLE OF PREGNANCY AND ESTRADIOL IN UTERINE ARTERY REMODELING

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Pregnant women at high altitude have lower circulating levels of estradiol than women at lower altitudes (Zamudio, JSGL, 1994). Differences in estradiol level may effect the normal vascular proliferative response to pregnancy. Normally, uterine artery (UA) diameter and wall thickness double during pregnancy. The mechanisms of this vascular remodeling are not well understood. We hypothesized that pregnancy stimulated DNA synthesis in all layers of the uterine vessels and that estradiol in part mediated this effect. We further hypothesized that increased DNA synthesis in SMOOTH MUSCLE CELLS (SMC) isolated from pregnant vs. nonpregnant guinea pigs would be detectable in culture and that the enhanced growth would be dependent on estradiol. Non-pregnant and pregnant guinea pigs were implanted with a thymidine analogue, bromodeoxyuridine (BrdU, 400 mg), for 14 d. Ovariectomized animals were implanted with BrdU and estradiol (2.5mg/14 d). Vessel sections were stained immunohistochemically with an anti-BrdU monoclonal antibody. Replication indices were calculated as the percent of BrdU-labeled nuclei from 600-1000 cells. Pregnancy increased DNA synthesis in the adventitia, media and intima of the uterine artery, radial artery, and uterine vein. Circulating estradiol levels correlated with the replication indices in the UA adventitia and all layers of the radial artery. Two week treatment of ovariectomized animals with estradiol increased DNA synthesis in the radial artery adventitia and tended ($p=0.08$) to increase replication indices in the media of all vessels examined. Growth responses were then measured *in vitro* using ³H-thymidine incorporation and cell counts. SMC from pregnant animals had increased spontaneous DNA synthesis and responsiveness to PDGF. This increased response to PDGF was reproduced in cells from non-pregnant animals by pre-treatment with estradiol. We concluded that pregnancy stimulates DNA synthesis in all layers of uterine vessels and that estradiol likely plays a role in regulation of pregnancy-induced vascular remodeling.

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EFFECTS OF ENDURANCE TRAINING UNDER ACUTE HYPOBARIC HYPOXIA ON HDL CHOLESTEROL CONCENTRATION

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The purpose of this study was to elucidate the effects of endurance training under acute hypobaric hypoxia on HDL cholesterol concentration. Three different groups of subjects participated in this training. Two groups were climbers (18 males and 3 females: 20-66 yrs) who intended to climb the high altitude mountains (7,560 m and Qomolangma), and other group was healthy adults (10 males and 1 female: 20-65 yrs) who designed to stay high altitude (>5,000m) for a month. The endurance training under acute hypobaric hypoxia by using a hypobaric simulator was a bicycle ergometer exercise with intensity 50 % VO_{2max} measured at sea level for 30 - 60 min. The simulated high altitudes adopted in this study were 3,500 m, 4,000m, 4,500 m, 5,000 m, 5,500 m, 6,000 m and 6,500 m. The trainings were done by once or twice a week for approximately two months. The blood samples were withdrawn before and after the training. The averaged total training number was 7.6 times. HDL cholesterol in the first group significantly increased from 52.8 ± 9.8 (mean \pm SD) to 66.2 ± 17.7 mg/dl ($p<0.05$). The changes of HDL concentration after the training in the Qomolangma group was not significant, but increased 4.8 mg/dl in average compared to the initial level. The HDL changes in the third group indicated significant increase from 44.5 to 51.0 mg/dl ($p<0.01$). These results suggested that the endurance training under acute hypobaric hypoxia may produce much HDL increase compared to that observed under normoxia.

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HYPERBARIC PROPHYLAXIS OF ACUTE MOUNTAIN SICKNESS

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Acute mountain sickness (AMS) may develop in sojourners to altitudes above 3,000m. Hypobaric hypoxia appears to be responsible for the onset of AMS in otherwise healthy mountaineers and recreational visitors to altitude. The purpose of this study was to determine if intermittent hyperbaric episodes would be effective in the prevention and/or reduction in the severity of altitude illness symptoms during a climbing expedition to Volcan Marmalejo, Chile (6,429m). Three randomly selected males of a twelve member climbing team received one hour of pressurization per day (140mmHg-ambient atmospheric pressure) in a multi-person portable hyperbaric chamber at camps above 3,000m. The 1993 Lake Louise Mountain Sickness Scoring System combined with SaO₂ (Nellcor N-20P pulse oximeter) were used to assess the degree of AMS. The results of this field study indicate that the subjects which received periodic hyperbaric therapy during the expedition had increased SaO₂ and reduced AMS symptomatology following each compression episode. As well, a higher percentage of subjects receiving hyperbaria reached the summit when compared with climbers that did not use the chamber. Data from this investigation suggests that intermittent hyperbaria may improve the ability for climbers to withstand the hypoxic confrontation associated with high altitude expeditions.

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FATAL RHABDOMYOLYSIS WHILE MOUNTAINEERING.

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We treated two rare cases of heat stroke and acute rhabdomyolysis encountered while mountaineering. The clinical findings were complicated with severe acute renal failure and disseminated intravascular coagulation (DIC), respectively. Though heat-related disorders occur frequently in mountain medicine in summer, severe and fatal rhabdomyolysis due to heat stroke is rare. It was also noteworthy that both patients had received treatment with antipsychotic drugs including phenothiazine. Even in a moderate exercise activity such as mountain, the possibility of rhabdomyolysis should be considered for all subjects, especially those with a history of antipsychotic neuroleptics.

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Microgravitation and hypoxia in tissues.

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Microgravitation causes distinct changes in circulation of blood and microcirculation. We developed a method, which allows to determine pO₂ directly in tissue of men during space flight. In 372 determinations average pO₂ in hand tissues of male was 37 \pm 0.2 tor and in astronauts - 40 \pm 0.8 tor, the last being higher significantly. Intensity of O₂ consumption in tissues, determined by rate of pO₂ decrease with pressed vessels, was 13 \pm 0.3 tor/min at this site. Average pO₂ in tissues of astronauts decreased from 40 \pm 0.8 to 25 \pm 4.0 tor and O₂ consumption - from 13 \pm 0.3 to 7.7 \pm 1.3 tor/min ($P<0.001$) during space flights, which lasted 96, 140 and 175 days. After these flights both pO₂ level and O₂ consumption were still reduced for 1 week: 33 \pm 1.4 tor and 11 \pm 0.56 tor/min correspondingly. Rehabilitation of astronauts resulted in normalization of oxygen regimen in tissues during 1 week. Besides, special biochemical study was carried out of biological oxidation and bioenergetics directly in tissues homogenates and mitochondria of rats after 22 days of flights in biosatellites. Abrupt decrease of bioenergetics and its alteration in tissues was found distinctly. Detailed study of pO₂ and mechanisms of tissue respiration was carried out as well by simulating microgravitation with longterm hypokinesia in men and animals during many ground experiments, which lasted upto 1 year. Distinctive reduction of tissue pO₂, oxidation, bioenergetics and glycolysis was found in these experiments too. Thus, there are all reasons to believe, that tissue hypoxia of certain degree appears during longterm microgravitation and its simulating by hypokinesia, which can be normalized by afterflight rehabilitation.

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Problem of hypoxia and oxygen homeostasis in the organism tissues.

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O₂ tension in the organism's tissues is the final characteristic of oxygen homeostasis of the organism. Developed by us variants of methods of pO₂ determination in tissues allowed to reveal 2 basic regularities: 1) there is oxygentopography of pO₂ average levels in different organs and tissues, that is pO₂ indices are different; 2) rhythmic fluctuations are observed often, which is probably distinctive periodic training mechanism in tissues. Use of similar principles of interval training by hypoxia showed its exceptional efficiency in adaptation of healthy people and therapy of various diseases in many experiments (Kovalenko E.A., 1989, 1990, 1993, 1995). At the same time studies of pO₂ in tissues allowed to evaluate rate of hypoxia in tissues under different influences and in extreme levels of hypoxia during ascents in altitude chambers in particular. Thus rates of tissue hypoxia in 24 climbers were determined during their ascent to the Everest in 1982. During their climbing to 7,8,9 and 10 km in altitude chamber pO₂ in hand skin was decreased from 36 \pm 1.1 to 12 \pm 0.6, 10 \pm 0.5, 8 \pm 0.9 and 6 \pm 0.45 tor at an average correspondingly, that is tissue pO₂ determination was succeeded for the first time at extreme altitudes of possible presence of men. Study of rate of tissue hypoxia under intensive hyperventilation during even 5 min was a new direction in investigations. It was found that in spite of increase in arterial pO₂ from 92 \pm 1.6 to 117 \pm 3.5 tor and decrease of blood pCO₂ from 40 to 20 tor, abrupt reduction of pO₂ took place in brain cortex (for 51%), liver (48%), kidney (47.5%), etc. Total average decrease of pO₂ in 28 tissues of the organism was 44.2%. Thus we could show for the first time, that hypoxia in various tissues could take place even with increase of arterial pO₂ and significant decrease of pCO₂ (for a half) during hyperventilation. In future experiments we succeeded to show, that adding of 5% of CO₂ to usual air could increase pO₂ significantly in different tissues both in normal ones and in hypoxia. Thus rate of pO₂ level in tissues directly is the most important index of real and final degree of hypoxia in the organism (Kovalenko E.A., 1990, 1993, 1995).

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EFFECT OF THE POIKILOCAPNIC-HYPOXIC INTERACTION ON PHYSIOLOGICAL TREMOR IN HUMANS

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We have postulated that acute exposure to hypoxia activates the 8-12 Hz oscillations of physiological tremor in humans via peripheral chemoreceptor stimulation of somatosensory somatosynaptic outflow. The objective of this study was to determine whether hypoxia also acted via a central mechanism to promote tremor. Given that peripheral chemoreceptor stimulation is less during hypoxic hypoxia (hH) than eupoxic hypoxia (eH), we hypothesized that hypoxic tremor would be less during hH than eH. Mechanical 8-12 Hz tremor was recorded with an accelerometer taped on the right index finger. Twelve male subjects were studied during hypoxic hypoxia, eupoxic hypoxia, hypoxic normoxia, and eupoxic normoxia. Using repeated-measures analysis of variance, six of twelve subjects (50%) showed greater tremor during hH than eH ($P<0.05$), thus demonstrating a positive poikilocapnic-hypoxic interaction (hH>eH). Therefore, in addition to the peripheral chemoreflex contribution to hypoxic tremor, we conclude that hypoxic tremor is also mediated by a central mechanism in some subjects. Despite similar end-tidal gases, those subjects demonstrating a positive poikilocapnic-hypoxic interaction also showed a relative hypoxenation compared to those possessing no or negative interaction (hH<eH). Least-squares analysis of $P_{\text{ET}}\text{O}_2$, $P_{\text{ET}}\text{CO}_2$, and the percent increase in V_a grouped by interaction is given below.

Interaction	$P_{\text{ET}}\text{O}_2$ (mmHg)	$P_{\text{ET}}\text{CO}_2$ (mmHg)	ΔV_a (%)
	hH	eH	hH
hH>eH (n=6)	44	44	37
	42	42	42
(mean \pm SEM)	± 3.2	± 2.9	± 1.4
hH<eH (n=6)	46	46	37
	43	43	43
(mean \pm SEM)	± 1.6	± 1.6	± 0.6

Significance: $P=\text{NS}$ vs. hH; $P=\text{NS}$ vs. eH; $P<0.05$ vs. hH>eH.

Central mechanisms which might contribute to hypoxic tremor include the supplemental influence of brain hypoxia due to a relative reduction in oxygen delivery from hypoxic cerebral vasoconstriction, or the modulating influence of sympathetic efferent activity subject to phasic inhibition by increased respiratory drive.

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ATRIAL NATRIURETIC PEPTIDE (ANP) SELECTIVELY INHIBITS HYPOXIC PULMONARY VASOCONSTRICION IN CONSCIOUS BEAGLE DOGS

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ANP has vasorelaxing properties. We tested the hypothesis, that ANP decreases the pulmonary vascular resistance during acute hypoxia. Ten chronically tracheotomized beagle dogs maintained under controlled conditions of sodium and water intake (2.5 mmol and 91 ml kg^{-1} body wt day $^{-1}$) were used in the following experiment: All dogs breathed spontaneously during the experiments. Arterial (MAP) and pulmonary arterial pressures (MPAP) were determined after 30 minutes of hypoxia (fraction of inspired oxygen (F_1O_2) = 0.1; arterial partial pressure of oxygen (p_1O_2) = 35 \pm 1 mm Hg) and after 30 minutes of hypoxia (p_1O_2 = 34 \pm 2 mm Hg) with simultaneous infusion of ANP (50 ng kg^{-1} body wt min $^{-1}$). Systemic (SVR) and pulmonary (PVR) vascular resistances were calculated with standard formula. Cardiac output was 2.2 ± 0.2 l min $^{-1}$ with hypoxia and 2.2 ± 0.2 l min $^{-1}$ in hypoxia with ANP infusion.

	MAP [mm Hg]	SVR [dyn s cm $^{-5}$]	MPAP [mm Hg]	PVR [dyn s cm $^{-5}$]
$\text{F}_1\text{O}_2=0.1$	111 \pm 8	4198 \pm 338	26 \pm 1	764 \pm 69
$\text{F}_1\text{O}_2=0.1+\text{ANP}$	108 \pm 7	3951 \pm 215	20 \pm 1 *	544 \pm 56 *

All values given are means \pm SEM. * $p<0.05$ hypoxia with vs. hypoxia without ANP infusion. In hypoxia, PVR decreased when exogenous ANP is administered at borderline physiological concentrations. This finding again emphasizes the well known differences in vascular regulation of the pulmonary and the systemic circulation. Whether or not it may give rise to a therapeutic approach for selectively decreasing elevated pulmonary artery pressure, has to be established however.

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WILL INERT FLUOROCARBON GASES TELL US INTRACELLULAR OXYGEN CONCENTRATIONS?

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Because oxygen is paramagnetic, when it is dissolved in fluorocarbon liquids, it relaxes the nuclear magnetic resonance (NMR) of the fluorine and one can measure the oxygen concentration by the fluorine NMR relaxation. We would like to use a similar trick for measuring oxygen inside cells, especially brain cells, but lipid perfluorocarbons will not cross the blood-brain barrier. Currently there is no non-invasive way to measure intracellular oxygen in brains. In addition, if one inserts an oxygen electrode into a brain, the measurement is likely to be too low because oxygen electrodes consume oxygen and disrupt the local blood supply. Perhaps there is a fluorocarbon gas that a subject can breath, that will cross the blood-brain barrier, and whose NMR relaxation will tell us how much oxygen is present. It should be soluble enough that we can detect it in the brain, but not preferentially soluble in membranes (to avoid anesthesia) and to obtain ample information from the cytosol). In addition, we would prefer that it has only one fluorine NMR spectral peak. Difluoromethane was a good first candidate. We measured its NMR relaxation as a function of PO_2 while dissolved in water and in olive oil. It was encouraging that the fluorine NMR relaxation times changed by 10% with a 100 mmHg change in PO_2 , but we need a greater change for measuring intracellular oxygen because the measurement precision is 5%. Perhaps a molecule with more carbon and fluorine will have a greater change in relaxation!

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UP-REGULATION OF CARDIAC α -ADRENERGIC RECEPTORS (α -AR) IN RATS EXPOSED TO PROLONGED HYPOXIAF. León-Velarde^{1,2}, J-P Richardet², G. Molinatti² and B. Crozier³
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Although mediated by different effector enzymes and second messengers, β -AR and α -ARs are involved in the cardiac positive inotropic effects. This study was performed to elucidate whether the modulation of β -AR by α -AR cardiac receptors observed in different experimental models is also present in the rats exposed to 21 days of hypoxia (380 Torr). In normoxic (N, n=6) and hypoxic rats (H, n=6), the weight ratio Right ventricle (RV)/Left ventricle (LV), the density (B_{max} , fmol/mg prot) and K_d (nM) of α -AR, and the capacity of norepinephrine (NE) to displace ^3H -prazosin were studied. Hypoxic rats showed, as expected, RV hypertrophy (RV/LV: H: 0.5 \pm 0.02; N: 0.25 \pm 0.01; $p<0.0001$). B_{max} in LV was higher in H rats when compared with N rats (H: 44.1 \pm 18; N: 22.8 \pm 7; $p<0.02$), however, no change was found in K_d (H: 0.037 \pm 0.007; N: 0.037 \pm 0.005). In RV, no difference was found in B_{max} and K_d between both groups. In H rats, LV showed a higher B_{max} (RV: 34.1 \pm 18; LV: 44.2 \pm 8; $p<0.0001$) and a lower K_d (RV: 0.037 \pm 0.005; LV: 0.11 \pm 0.01; $p<0.0001$) than RV. The displacement curves showed that α -AR have a significant higher specific binding for ^3H -prazosin (SB) (for concentrations higher than 10^{-9}M of NE) in the H rats when compared with N rats. In N rats, no difference was found in SB between RV and LV, whereas in H rats RV have a higher SB when compared with LV. In conclusion, the present data support the α -AR up-regulation hypothesis as one of the mechanisms of myocardial response to prolonged hypoxia. Additionally, the higher SB in RV of H rats, may indicate that the stimulus responsible for cardiac hypertrophy may include α -AR modulation.

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ABSTRACTS

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EFFECTS OF HYPOXIA ON THE LEVELS OF HYPOTHALAMIC ACETYLCHOLINE IN POSTNATAL DEVELOPMENT RATS AND *MICROTUS OECONOMUS* *

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We investigated the effect of hypoxia on the levels of acetylcholine (Ach) in postnatal 1d (P1), 7d (P7), 14d (P14), 21d (P21), 28d (P28) rat and 5d (P5), 14d (P14), 25d (P25), 120d (P120), 390d (P390) *Microtus oeconomus* (MO) hypothalamus and MO blood. The results showed that: 1) The levels of Ach in postnatal development rat and MO hypothalamus and MO blood was no changed; 2) Acute hypoxia for 24h has different effect on the levels of Ach in postnatal development rat hypothalamus: 1) decreased by 6.50% ($P<0.05$) and 10.43% ($P<0.01$) under 5km and 7km hypoxia in P1 hypothalamus, respectively. It was no significant change on P7 under 5km and 7km acute hypoxia. It decreased by 16.47% ($P<0.05$) under 7km acute hypoxia, but 5km acute hypoxia was no significant effect in P14 hypothalamus. Nevertheless, It increased by 22.37% ($P<0.05$) and 38.20% ($P<0.05$) on P21 and P28 under 7km acute hypoxia, respectively. But 5km acute hypoxia was no significant effect. The rat after neonatal 3 days developed for 1, 7, 14, 21, 28 days in simulated hypoxic chamber. The levels of Ach in rat hypothalamus decreased by 32.69% ($P<0.01$) and 23.30% ($P<0.01$) only on 1, 14 days. Other days was no significant change. It was suggested that hypoxia inhibited neonatal rat hypothalamic cholinergic neurons activity, but stimulate on P21, P28; 3) The levels of Ach in neonatal MO hypothalamus and blood decreased by 23.83% ($P<0.01$) on P5 hypothalamic Ach and by 43.52% ($P<0.05$), 19.23% ($P<0.05$) on P14, P25 MO blood. Ach under 7km acute hypoxia, respectively. Which Suggested that hypoxia inhibits the activity of neonatal MO parasympathetic nerve system, but not in adult and aged. The above result suggested that hypothalamic cholinergic neurons has different ontogenesis model owing to species on postnatal development and under hypoxia.

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EFFECTS OF HYPOXIA ON THE LEVELS OF HYPOTHALAMIC NOREPINEPHRINE (NE) IN POSTNATAL DEVELOPMENT RAT AND *MICROTUS OECONOMUS* *

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We investigated the effect of hypoxia on the levels of hypothalamic norepinephrine (NE) in postnatal 1d (P1), 7d (P7), 14d (P14), 21d (P21), 28d (P28) rat and 5d (P5), 14d (P14), 25d (P25), 120d (P120), 390d (P390) *Microtus oeconomus* (MO). The results showed that: 1) The levels of hypothalamic NE in postnatal development rats increased with developmental age before 28d, but in hypothalamus of MO NE was not changed; 2) Acute hypoxia for 24h has different effect on the levels of hypothalamus of NE in postnatal development rats: It increased in P1 NE by 27.37% ($p<0.05$) and 45.25% ($p<0.01$) under 5km and 7km hypoxia, respectively. NE was no significant change in P7 under 5km and 7km, NE increased by 27.38% ($p<0.01$) and 48.76% ($p<0.001$) under 5km and 7km in P14 rat, P21 decreased by 41.90% ($p<0.01$) and 102.79% ($p<0.001$) under 5km and 7km, respectively. NE decreased by 30.17% ($p<0.001$) under 7km hypoxia, but 5km did not affect NE. Neonatal rats 3 days old developed for 1, 7, 14, 21, 28 days in simulated hypoxic chamber of 5km hypoxia, the levels of hypothalamic NE increased by 27.37% ($p<0.05$) in 1d and 42.76% ($p<0.05$) in 14d, no changes in remained group. In summary, hypoxia stimulate hypothalamic NE neurons activity in neonatal rat, but inhibit it in P21 and P28; 3) The levels of hypothalamic NE increased by 41.85% ($p<0.05$) in neonatal P5 MO during 7km acute hypoxia, was no significant change in other group, indicating that NE neurons in hypothalamus of baby MO are much sensitive to hypoxia. All the data above indicated that NE neurons activities and response to acute and chronic hypoxia in postnatal development are different with different of species between rats and MO.

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IS THE INCREASE IN VENTILATION WITH HYPOXIA AFFECTED BY DENSITY?

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Exercising humans and horses increase ventilation (\dot{V}_E) when breathing low density helium-O₂ mixtures instead of air. These mixtures acutely aid patients with obstructive lung disease by lowering arterial PCO₂, implying a higher \dot{V}_E , but VCO₂ and \dot{V}_E are rarely reported. Differences in \dot{V}_E have been reported at the same P_{O₂} between altitude and normobaric hypoxia, where density is 50% higher. We wished to clarify how two hypoxic gas mixtures with differing densities would affect \dot{V}_E in healthy subjects and whether the differing densities would be altered by resistive airway loading. Reduced density can reduce the metabolic cost of breathing and result in increased PO₂, which will reduce the hypoxic stimulus and thereby attenuate the increase in \dot{V}_E . In 10 subjects, gas exchange measurements were made from 12 to 15 min after the onset of breathing three different gases (air, HxN₂, 13.7%O₂ in N₂ and HxHe: 13.7%O₂ in helium) with 10 min of free breathing between the three gases. These measurements were repeated on another day with the external airway constricted such that both inspiratory and expiratory airway resistance (R_A) was increased to 27cm H₂O s⁻¹ l⁻¹ on air. Without imposed resistance, \dot{V}_E increased +25% from air on both hypoxic mixtures as P_{O₂} was reduced from 122 to 80mmHg. Loaded breathing significantly reduced frequency and increased tidal volume compared to no load for all three gases and the \dot{V}_E was the same on air as without loading, but no increase in \dot{V}_E occurred with HxN₂ or with HxHe. With HxHe the R_A was 65% lower and the breathing frequency increased and tidal volume decreased toward the non-loaded pattern. A significant positive relationship between VCO₂ and calculated work of breathing in the 3 loaded measurements ($n=30$, $r=+0.65$) indicated a breathing efficiency of 3%. These results demonstrate that imposed R_A prevented the acute \dot{V}_E response to hypoxia. The response did not return with a reduction in gas density and R_A , suggesting that the threshold for the inhibition of chemoreceptors by mechanoreceptors was still exceeded.

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A-V DIFFERENCE IN LACTATE IS NOT DEPENDENT ON VENOUS PO₂ DURING EXERCISE

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During incremental exercise with a small muscle group we were able to show that lactate concentration and A-V difference in [Lac] are not dependent on venous PO₂. In this study we combined forearm and cycling exercise in a way that the forearm could be regarded as a large muscle group. **Methods:** 8 Male subjects performed forearm exercise at 65% (frequency 24/min) of their maximal workload reached in an incremental exercise test. After ten minutes of forearm exercise (pre) they started cycling at 60% of W_{max} of an incremental exercise test for ten minutes while continuing with the forearm exercise (com). Cycling exercise was stopped after 10 min, forearm exercise continued for another ten minutes (post). In cubital venous blood PO₂, SO₂, PCO₂, Hb, pH and lactate were measured. Additionally lactate was measured in arterialized blood from an earlobe. Forearm blood flow was measured plethysmographically. a-VO₂ was calculated assuming an oxygen saturation of about 95% in the arterial blood. **Results:** During pre a-VO₂[Lac] increased from -0.5 to -2.8 +/- 0.7 mmol/l after 3 minutes. In spite of a decreasing PO₂ lactate release decreased within the next 7 minutes. In spite of the increasing arterial [Lac] a-VO₂[Lac] increased during the first 6 min of com. At the end of post a-VO₂[Lac] almost reached preexercise values (-0.6 +/- 0.9 mmol/l). Under none of these conditions a significant relationship between a-VO₂[Lac] and PO₂ or a-VO₂ in oxygen could be found. **Conclusion:** Lactate release is not related to PO₂ under these conditions. Thus production and/or release must be governed by other factors. One of these factors might be muscle blood flow. But especially during pre and post a-VO₂[Lac] was not related to blood flow. Therefore the decrease in lactate release might reflect the "switches" between metabolic pathways during the time exercise being performed.

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IS BIOSORBIN MCT SUITABLE AS FOOD DURING EXPEDITIONS
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Peroni used Biosorbin MCT (Pfriemer Nutricia) several times as solitary food during expeditions. He postulated that the caloric expenditure might be reduced by about 25 % while using biosorbin. The following experiments were performed to test this hypothesis. **Methods:** 8 subjects performed endurance tests with 50% of their maximal power reached in an incremental cycle ergometer test. After 15 and 34 min power was increased to 80 % for 4 min. The exercise test was performed before and after the 7 days lasting diet. Oxygen uptake, CO_2 output, lactate, heart rate, glucose, triglycerides, free fatty acids were measured in cubital venous during the test. Urea in blood was measured before the exercise tests. **Results:** RQ was reduced during rest and exercise after the diet ($p<0.01$). The calculated contribution of the fat metabolism increased from about 25 to 32 %. As exercise was started after the diet plasma FFA were enhanced. FFA decreased to the control values during exercise. After the diet glucose concentration was not affected, lactate tended to be lower. Urea was enhanced by about 24% ($p<0.01$). Body weight was reduced slightly. **Conclusion:** As the changes are in part typical for reduced caloric intake, the consumption of biosorbin seems not to reduce energy expenditure during normal daily activity. During exercise oxygen uptake is the same, thus the caloric need is similar. But if the intake is equivalent to the expenditure biosorbin may serve as an adequate food at least for strenuous tours. Under these conditions the beneficial effect of MCT as a glycogen saving fuel during exercise might become more prominent.

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VENTILATORY RESPONSE TO SUSTAINED HYPOXIA IN SUBJECTS SUSCEPTIBLE TO HIGH-ALTITUDE PULMONARY EDEMA
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To investigate the role of abnormal control of ventilation in the pathophysiology of high-altitude pulmonary edema (HAPE), we evaluated the ventilatory response to moderate (arterial oxygen saturation $80 \pm 2\%$), sustained, isocapnic hypoxia at low altitude (610m, barometric pressure 700 Torr) in eight subjects with histories of high altitude pulmonary edema (HAPE-S) and in five subjects who had been asymptomatic during previous altitude exposure. During 25 min of hypoxia, an initial increase and subsequent gradual decline in ventilation was observed in both groups. In HAPE-S, however, the magnitude of the ventilatory response was significantly lower than that of control groups (analysis of variance with repeated measures, $p<0.05$). The lower ventilatory response during sustained hypoxia in HAPE-S correlated confidently with their lower acute ventilatory response (HVR) compared to that of control subjects. The gradual ventilatory decline during sustained hypoxia (hypoxic ventilatory depression; HVD) was similar between HAPE-S and control subjects (2.38 ± 0.97 L vs 3.34 ± 0.95 L, respectively; NS). These results suggest that the hypoxilation observed in HAPE-S during sustained hypoxia may be attributable both to a lower HVR and HVD. The exact role of HVD in the development of high-altitude pulmonary edema needs further study.

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WOMEN AT ALTITUDE: THE RELATIONSHIP BETWEEN FLUID BALANCE AND ACUTE MOUNTAIN SICKNESS OVER THE MENSTRUAL CYCLE
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Many studies demonstrate an inverse relationship between symptoms of acute mountain sickness (AMS) and the degree of diuresis upon acute exposure to high altitude. This study was designed to investigate this relationship in women while controlling for menstrual cycle phase. Sixteen subjects (age: 21.7 ± 0.5 yrs; ht: 167.4 ± 1.1 cm; wt: 62.2 ± 1.0 kg) were transported to Pikes Peak (4301 m) in the beginning of either the follicular (F) or luteal (L) phase of the menstrual cycle. Fluid input and urinary output were measured daily throughout the 12 day study period. Cerebral symptoms of AMS (AMS-C) were assessed twice a day using the Environmental Symptoms Questionnaire (ESQ). Relative to fluid input, daily urinary fluid losses were greater during the first two days of altitude exposure as compared to days 3-11 ($p<0.05$). AMS-C scores were significantly greater for the first two days of exposure (mean: 0.89 , SD: 0.54) than for days 3-11 (mean: 0.22 , SD: 0.21) ($p<0.01$). Net fluid losses during days 1 and 2 for subjects with AMS-C scores above 0.7 (mean AMS-C: 1.2 , SD: 0.4) were not significantly different than losses for subjects with AMS-C scores below 0.7 (mean AMS-C: 0.4 , SD: 0.1). There was no independent effect of cycle phase upon urinary fluid losses. These data suggest that net fluid loss upon initial exposure to high altitude is not inversely related to symptoms of AMS in this population. This study was supported by the Department of Defense #DAMD-17-95-C-5110.

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PRESERVED DIFFUSION CAPACITY DURING EXERCISE IN HYPOBARIC HYPOXIA (418mmHg)
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In hypobaric-hypoxia, diffusion limitation of oxygen, as well as advanced V_a/Q mismatching, is believed a major factor inducing elevated $A-a\text{DO}_2$ during exercise. The purpose of this study is to measure pulmonary diffusing capacity directly during hypobaric-hypoxic exercise. Subjects are 13 healthy volunteers. Steady state exercise test for three minutes at 0%, 25%, 50%, 62.5% and 75% of the maximum work rate were conducted at PB=760mmHg and in a hypobaric chamber (PB=418mmHg). During the last minute of each exercise period, we calculated DL_{CO} , Qc , Qt , CvO_2 and VO_2 by rebreathing gas method of 0.3% C^{18}O , 0.65% C_2H_2 , 9.5% Ar , 25% O_2 with supplemented N_2 by a mass spectrometer (Westron RL600, Japan). 1. DL_{CO} at a given work rate at 418mmHg exceeded those at 760mmHg up to 62.5% level, where subjects reached exhaustion. 2. No differences were found in the slope of DL_{CO} vs. Qc . 3. Though there was no difference in $e^{\text{DL}_{\text{CO}}(418)}$ vs. VO_2 , subjects who showed blunted $e^{\text{DL}_{\text{CO}}(418)}$ at 418mmHg exercise revealed less performance at high altitude trekking. 4. After exhaustion at 418 mmHg, DL_{CO} turned to be markedly depressed with mild reduction in Qc . These results suggest that lung diffusion capacity was not impaired but rather augmented during moderate to sub-maximum exercise at 418mmHg. Diffusion limitation was not considered to be a major factor limiting maximum VO_2 at 418mmHg. Measurement of $e^{\text{DL}_{\text{CO}}(418)}$ vs. VO_2 in hypobaric chamber may predict a part of high altitude performance.

ABSTRACTS

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THE STEADINESS OF WOMEN TO ACUTE AND CHRONIC HYPOXIA

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The steadiness of the women to acute hypoxia and the peculiarities of their adaptation to chronic hypoxia are studied not enough. Our aim was the comparison of adaptive reactions to hypoxia of men and women. We examined 4 healthy women and 6 men in normal conditions, on 1-2 and 7-8 days ($h=2200m$) and on 17-18 days ($h=4200m$) in the mountains of Caucasus. Pneumogram, ECG, EEG and the indices of blood were registered. Before the mountains and after the return the steadiness to altitude was determined in the decompression chamber during the stepped rise up to the permissible high altitude. The results showed that in the mountains the dynamics of the researched parameters in men and women was significantly similar. But the interruptions of the respiratory rhythm in women ($h=4200m$) were more distinct. In the decompression chamber before and after the mountains the women reached the more high altitudes, than men. So the mechanisms of adaptation to hypoxia in men and women are similar. But the women are comparatively more steady to acute hypoxia. At the same time according to cardiorespiratory responses the female organism is more sensitive to chronic hypoxia

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DIFFUSION LIMITATION DURING EXERCISE IN PATIENTS WITH INTERSTITIAL FIBROSIS

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This study was designed to investigate the influence of exercise on pulmonary diffusion capacity (DL_{CO}), capillary blood flow (Q_c) and diffusion limitation in patients with interstitial fibrosis (IF). Ten patients with idiopathic pulmonary fibrosis and interstitial fibrosis associated with collagen vascular disease and eight non-smoking healthy normal controls (N) were examined by steady-state exercise test on a bicycle ergometer at the 0%, 25%, 50%, 62.5% and 75% of maximal load determined by step incremental protocol beforehand. During the last minute of each 3 minutes exercise, they rebreathed mixed gas (C₂H₂:0.65%, Ar:9.5%, O₂:25%, C₁₈O:0.31%, N₂balance). Q_c, DL_{CO}, and VO₂ were calculated from breath-by-breath measurement of C₂H₂, C₁₈O, and O₂, respectively, after correction for gas mixing delay using Ar by a mass spectrometer (Westron RL-600, Japan). 1) Q_c and DL_{CO} were linearly increased in accordance with VO₂ in both groups. 2) No significant differences were seen in $\Delta Q_c / \Delta V_{O_2}$ and $\Delta DL_{CO} / \Delta V_{O_2}$ between two groups. However, $\Delta DL_{CO} / \Delta Q_c$ in IF was significantly smaller than in N. 3) The slope of e^{-DL_{CO}/Q_c} vs. VO₂ in IF was steeper than that in N. 4) $\Delta(e^{-DL_{CO}/Q_c}) / \Delta V_{O_2}$ was correlated with % DL_{CO}(SB) at rest. These results suggest that though diffusion capacity itself seems to be intact, diffusion limitation becomes deteriorated during exercise in IF patients.

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REGULATION OF GLYCOGENOLYSIS AND PYRUVATE OXIDATION IN HUMAN SKELETAL MUSCLE DURING INTENSE EXERCISE

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The purpose of this study was to determine the time course for the transformation of glycogen phosphorylase and pyruvate dehydrogenase (PDH) during the first and third bouts of maximal intermittent exercise. Six subjects completed three 30-s bouts of maximal isokinetic cycling at 100 rpm, each separated by 4 min of rest. Biopsies of the vastus lateralis were taken before, and at 6s, 15s and 30s of bouts 1 and 3. Total work in bout 1 was 18.4 \pm 0.5 kJ, and decreased to 13.7 \pm 1.0 kJ in bout 3. Prior to bout 1, PDH was 14.2 \pm 0.1 % activated, increased to 48.4 \pm 0.1 % at 6s, and was totally activated by 15s. The total fraction of active phosphorylase (PHOS g) was 9.9 \pm 2.2 % at rest, increased to 46.8 \pm 5.3 % and 47.4 \pm 6.4 % at 6s and 15s, respectively, and by 30s, decreased to 21.8 \pm 4.2 %. Prior to bout 3, PDH was 41.7 \pm 0.1 % activated; by 6s it was 100% activated. PHOS g fraction increased from 10.5 \pm 2.7 % to 14.7 \pm 2.7 % and 20.5 \pm 1.6 % at 6s and 15s, respectively, and was partially inactivated to 16.2 \pm 5.7 % at 30s. Glycogen utilization was 127.0 \pm 35.1 and 15.1 \pm 10.2 mmol/kg dw in bouts 1 and 3, respectively. Lactate increased from 2.7 \pm 0.4 to 76.1 \pm 7.4 mmol/kg dw in bout 1 and from 97.7 \pm 16.7 to 107.1 \pm 15.3 mmol/kg dw in bout 3. In bout 1, the rapid transformation to PHOS g was associated with rapid increases in glycogenolysis and a large accumulation of lactate due to the delayed activation of PDH. However, in bout 3 the small change in PHOS g resulted in a lower rate of glycogenolysis and the rapid activation of PDH resulted in a greater oxidation of pyruvate and hence less lactate accumulation.

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NO EVIDENCE FOR INFLAMMATORY REACTIONS DURING THE FIRST 24 HOURS OF HYPOBARIC HYPOXIA EQUIVALENT TO 4000m IN SUBJECTS SUSCEPTIBLE TO HIGH ALTITUDE PULMONARY EDEMA

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In order to investigate the role of inflammatory reactions in the development of high altitude pulmonary edema (HAPE), 7 HAPE susceptible subjects (HAPE-S) and 5 controls (C-S) were exposed to simulated altitude of 4000m for 24h in a hypobaric chamber. Acute phase proteins [i.e. complement C3 (C3C), α_1 -antitrypsin (α_1 A), transferrin (TRF) and α_1 -reactive protein (CRP)] and interleukin-6 (IL-6), an inflammatory mediator, were measured before ascent at Zurich level (450m) and after 20h at 4000m in venous blood. packed cell volume (PCV), total protein (TP), albumin (ALB), erythropoietin (EPO) and vascular endothelial growth factor (VEGF) were also determined before ascent (that lasted 4h), after 6h at 4000m and after 20h at 4000m. All blood samples were taken from an antecubital vein. Peripheral arterial oxygen saturation (SaO₂) was continuously recorded with a pulse oximeter. From variations in PCV relative plasma volume changes were calculated, and concentrations were corrected for EPO was significantly higher after 6h of hypoxia ($p<0.05$), increased further and correlated well with mean SaO₂ values (10 means) in both groups. VEGF did not show any significant increase. C3C and α_1 A increased slightly ($p<0.05$) in HAPE-S but remained in normal clinical ranges (<1.2 g/l resp. <2 g/l) and were not significantly different from C-S. TRF, CRP, TP/ALB ratio as an indicator for dysproteinemia and IL-6 remained unchanged. We conclude, that during the first 20h at high altitude (4000m) there is neither any evidence for a contribution of inflammation to the development of HAPE, nor an indication in peripheral venous samples, that VEGF plays a role in increasing capillary permeability.

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QUALIFICATION OF THE VIDAS® SYSTEM AT 4350 METERS
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The VIDAS® (Vitek ImmunoDiagnostix Assay System) is a compact automated immunoassay instrument. The goal of this experiment was to validate a system able to perform hormonal testing in a very high altitude laboratory. The parameters measured were LH, FSH, Prolactin, TSH, T3, T4, FT3 and FT4. The site chosen was the Vallois Observatory at 4,350 meters under the summit of Mont-Blanc. In collaboration with the ARPE, several experiments were conducted. In order to check the intra-assay precision samples were assayed 12 times on the same instrument in bioMéreux laboratory at an altitude of 200 m and in the Vallois Observatory at 4,350 m. For example for a LH sample at 8 mIU/ml, the Coefficient of Variation (CV) obtained was 2.2% in bioMéreux and 1.9% in Vallois observatory. For a LH sample at 47 mIU/ml, CV's were respectively 1.6% and 3.0%. In order to check the inter-assay precision, samples were assayed 10 times in different runs. For a LH sample at 8 mIU/ml CV's were respectively 2.7% and 2.4%. For a LH sample at 47 mIU/ml, CV's were respectively 1.9% and 1.7%. Sera previously tested in bioMéreux were then assayed in the Vallois Observatory. The linear regression obtained for LH samples (N=18) was: Vallois Value = 1.09 x Marcy Value - 0.33, R = 0.99. The instrument was then used to assay hormones on volunteers under TRH stimulation to induce Prolactin synthesis. No significant differences were shown on assays performed in Marcy and in Vallois. This compact automated instrument with its dedicated ready-to-use reagent worked perfectly in this high altitude environment. It confirms the possibility to use it for a long term experiments at high altitude in future missions.

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COMPARISON OF VENTILATORY RESPONSE TO HYPOXIA
 AND EXERCISE BETWEEN LOW AND HIGH ALTITUDE
 TIBETAN

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To examine differences between lifelong Tibetan residing of lower and high altitude areas in ventilatory response to hypoxia and exercise, we measured hypoxic ventilatory response (HVR) with a polioclampic method and exercise test with incremental bicycle ergometer in 18 lower-altitude Tibetan (2,260m) and 21 high-altitude Tibetan (4,300 to 4,700m) at Xining (2,260m), Qinghai, China. The studies were performed on the 10th day after the high-altitude Tibetan had descended to Xining from their altitude home. Both groups are considered of healthy young men with similar lifestyles and genetic background living at different altitude of Qinghai-Tibet plateau. The slope of the HVR in the lower-altitude Tibetans was significantly lower than that in the high-altitude Tibetans (AVE/ASA₀₂ : 0.46±0.04 vs. 0.81±0.07, p<0.01). At maximal efforts, high-altitude Tibetan, compared with lower-altitude Tibetan, had lower ventilation (68.2±2.1 vs. 77.3±3.5 l/min BTPS, p<0.05), lower oxygen uptake (2.28±0.1 vs. 3.56±0.10 l/min STPD, p<0.01) and lower heart rate (146.4±5.6 vs. 169.3±3.4 beats/min, p<0.01). These results demonstrated that ventilatory response to hypoxia and exercise was significantly lower in the high-altitude Tibetan than in the lower-altitude Tibetan. We conclude that ventilatory response to acute hypoxia is blunted in the high-altitude Tibetan, but not in the lower-altitude Tibetan, and imply that the attenuated respiratory sensitivity in the high altitude Tibetans is related to the high elevation of residence.

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BLUNTED HYPOXIC PULMONARY VASOCONSTRICITION
 RESPONSE IN PIKA AT HIGH ALTITUDE

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 To determine the possible mechanism of adaptation to chronic hypoxia on pulmonary circulation, we had direct measurements of pulmonary arterial pressure (Ppa) in 10 awake Pikas (Ochotona curzonoae), which were captured at 4,300m and then transported to Xining (2,260m), and in 10 Wistar rats in the large decompression chamber (simulated 4,300m and 5,000m) in Xining, Qinghai, China. Hemodynamic measurement was obtained after one hour each simulated altitude while the animals were still in the chamber. Histology and immunohistochemistry on lung tissues were also studied. It was found that the Ppa in Pika, after acute exposure to 4,300m and 5,000m, did not rise significantly, whereas in the rats it rose progress. The changes of Ppa from 2,260m to 4,300m and to 5,000m (ΔPpa) was 1.48±0.49 and 4.80±0.67 mmHg in Pika, and 10.58±3.36 and 19.10±2.28 mmHg in the rats, respectively. The ratio of right ventricular to left ventricular plus septal weight was 0.22 in the Pika and 0.45 in the rat. The percent wall thickness of small pulmonary arteries was 9.2% in the Pika and 27.2% in the rat, and it was well correlated with Ppa in the both groups ($r=0.78$). Lung mast cell (Toluidine blue staining) was observed only in the rats (7.1±0.33 cells/mm²). There was highly positive staining for mast cell tryptase with monoclonal antibody AAI (Dako-Mast Cell) and transforming growth factor-Beta 1 (TGF-β1) with monocloned mouse anti-TGF-β1 antibody in the perivascular and peribronchial tissues in the rats, whereas no demonstrable reaction was observed in the Pika. It is concluded that the Pika has adapted to high altitude by losing the hypoxic pulmonary vasoconstriction, the mechanism by which could be associated with some growth factor such as TGF-β1 and tryptase.

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CALCIUM CHANNEL BLOCKER NICARDIPINE DOES NOT
 IMPAIR GAS EXCHANGE AND EXERCISE PERFORMANCE
 IN ACUTE ALTITUDE HYPOXIA

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Calcium channel blockers are active against high-altitude pulmonary edema, partly by reducing pulmonary arterial pressure. However, these drugs have been shown to modify the distribution of pulmonary blood flow and may therefore enhance the intra-pulmonary shunt and favour hypoxemia, especially during exercise. The present study was designed to evaluate the effect of nicardipine on exercise performance and gas exchange after 48 hours of exposure to 4,350m. Ten sea-level natives (aged 24 - 34 yrs) performed a maximal exercise at sea-level (N), 24 h (H1) and 48 h (H2) after passive transport to Observatoire Vallois (4,350m). No treatment was administered in N and H1 conditions. Nicardipine (Ni, n=5) or Placebo (Pla, n=5) (20 mg) was administered orally in H2, 2 hours before maximal exercise. Subjects suffered from mild to moderate acute mountain sickness (AMS) but none of them showed clinical symptoms of pulmonary edema. $\dot{V}O_{2\text{max}}$ decreased by 22 % from N to H1. Variation in $\dot{V}O_{2\text{max}}$ from H1 to H2 was insignificant in both Ni and Pla group. $\dot{P}O_2$ from arterialized capillary blood, alveolo-arterial $\dot{P}O_2$ difference, ventilation, end-tidal PCO_2 , arterial saturation (oximetry), heart rate at submaximal or maximal exercise were not significantly influenced by the treatment. We conclude that acute administration of a calcium blocker does not impair pulmonary gas exchange and exercise tolerance at high altitude in subjects with mild to moderate AMS.

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THE INCIDENCE OF ACUTE MOUNTAIN SICKNESS AS AFFECTED BY HORMONAL CONTRACEPTIVES

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Despite progesterone's recognized effect as a ventilatory stimulant, its influence upon acute mountain sickness (AMS) has undergone extremely limited investigation. This study was designed to compare the incidence of AMS between the genders; between women using hormonal forms of contraception (oral contraceptives: Depo-Provera®, Norplant®) as contrasted with those who were not, and to compare the incidence of AMS between the stages of the female menstrual cycle. Using convenience sampling, 44 recreational climbers (35 men and 9 women), aged 18-52 years, were studied after a summit attempt on Mt. Rainier (3,072m). Participants completed the Lake Louise Consensus Questionnaire to quantify AMS occurrence. 40% of the men and 55% of the women were afflicted with AMS. Correlation of extrapolated menstrual cycle phase to AMS susceptibility was also attempted. Given the limited sample size of 9 women, neither the use of hormonal contraceptives nor the menstrual cycle stage was associated with increased or decreased AMS rates. Further elucidation of the effects of either endogenous or exogenous progesterone on AMS was not obtained.

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SERUM CARDIAC ENZYMES EXERCISE & ALTITUDE.

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Elevation of "cardiac-specific" serum markers of myocardial ischemia has been shown in asymptomatic marathoners after the race at sea level. We examined serum creatine kinase MB (CK-MB), myoglobin (MYGL), cardiac myosin light chains (MLC) and troponin complex I (TNI) in participants in the Sandia Wilderness Crossing, 46 K run reaching an altitude of 3300m. Nineteen runners were examined before and after the race in 1993 and 27 runners participated in 1995 donating blood before, after, 24 and 48 hours after the race. There were 9 non-running controls. The cardiac markers were measured by rapid format and reference assays. MYGL increased and 24 hours after the race it remained elevated. CK-MB increased and was highest immediately after the race. MLC increased and was highest immediately after the race. TNI was significantly different over all times ($P=0.003$) and highest immediately after the race. All measures were significantly different from controls. The increase in TNI was not related to any other "cardiac-specific" marker nor to age or finishing times. Thus "cardiac-specific" markers may not contribute to the diagnosis of myocardial infarction in well-trained ultrarunners and may have their origin in skeletal rather than myocardial muscle after an altitude ultra-race. Supported by grants from the NMHEMC Research Foundation and Istituto C. Mondino, Univ. of Pavia Italy.

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EFFECT OF EXERCISE-INDUCED HEMOCONCENTRATION ON PERFORMANCE AND NOREPINEPHRINE RESPONSE DURING REPEATED SPRINTS AT HIGH ALTITUDE

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Exercise-induced hemoconcentration increases at high altitude. We tested the hypothesis that, at high altitude, this mechanism is involved in 1) the limitation of performance during repeated sprints, and 2) the higher norepinephrine response. Twelve subjects (8 males, 4 females) performed four 20-s cycle sprints (Wingate test: WT), each separated by 4-min of passive recovery, in four conditions: at sea-level, without (SLC) and with the infusion of a plasma expander (6 ml kg^{-1}) during repeated WT (SLI), and after 6-7 days at 4,350 m following the same procedure (HAC and HA). Hematocrit (Hct), hemoglobin ([Hb]), plasma norepinephrine ([NE]), and lactate ([Lac]) concentrations were measured before and 1.5 min after repeated WT. Mean power output (MPO) during WT and total work over four repeated WT (W_{total}) were measured. Resting [Hb], but not Hct, was higher at 4,350 m than at sea-level ($n=10$). The decrease in plasma volume (calculated from Hct and [Hb]) induced by repeated WT was 19±4% (SL) and 17±4% (HA) without infusion and was reduced to 7±3% (SL) and 2±5% (HA) during infusion. The decrease in MPO from WT1 to WT4 was similar between SLC, SLI and HAC, and was greater in HAC. At sea-level, W_{total} was not modified by infusion (SLC: $655\pm121 \text{ J kg}^{-1}$, SLI: $664\pm125 \text{ J kg}^{-1}$). W_{total} was reduced ($p<0.02$) from SLC to HAC ($630\pm113 \text{ J kg}^{-1}$). Finally, W_{total} was higher ($p<0.005$) in HAC ($652\pm121 \text{ J kg}^{-1}$) than in HAC. At HA, the improvement in W_{total} with infusion was related to the correction of plasma volume decrease ($p<0.05$). Post-WT [Lac] was lower in HAC than in SLC ($p<0.05$). Post-WT [NE] was higher at HA than at SL but was not altered by infusion. These results therefore suggest that the reduction in blood or plasma volume 1) is a limiting factor of performance during repeated sprints at high altitude, but 2) does not account for the higher norepinephrine response observed at high altitude.

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HYPOMETABOLIC RESPONSE TO HYPOXIA OF CONSCIOUS ADULT RATS : EFFECT OF 2,4-DINITROPHENOL.

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During acute hypoxia, an hypometabolic response is commonly observed in many newborn and adult mammals. We hypothesized that pharmacological uncoupling of the oxidative phosphorylation could reverse the hypoxic drop in oxygen consumption (\dot{V}_{O_2}). Metabolic, ventilatory, and cardiovascular measurements were collected in conscious rats in air and hypoxia, before and after i.v. injection of the mitochondrial uncoupler 2,4-dinitrophenol (DNP). In hypoxia (10% O_2 breathing, 60% arterial O_2 saturation), \dot{V}_{O_2} , measured by an open-flow technique, was less than in normoxia (~80%). Successive DNP injections (6 mg/kg \times 4) progressively increased \dot{V}_{O_2} in both normoxia and hypoxia, by similar amounts. Body temperature did not change. The DNP-stimulated \dot{V}_{O_2} during hypoxia could even exceed the control normoxic value. A single DNP injection (17 mg/kg, iv) had a similar metabolic effect; it also resulted in hypotension and a drop in systemic vascular resistance. We conclude that pharmacological stimulation of \dot{V}_{O_2} can counteract the \dot{V}_{O_2} drop determined by hypoxia. Hypoxic hypometabolism is likely to reflect a regulated depression of thermogenesis, with no limitation in cellular O_2 availability. (MRC Canada)

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NEW ASPECTS OF THE VENTILATORY RESPONSE IN SUBJECTS SUSCEPTIBLE TO HIGH ALTITUDE PULMONARY EDEMA (HAPE) DURING A 24-HOUR EXPOSURE TO HYPOBARIC HYPOXIA EQUIVALENT TO 4000m

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In order to investigate the dynamics of the ventilatory response to sustained hypobaric hypoxia and their contribution to the pronounced arterial hypoxemia in subjects susceptible to high-altitude pulmonary edema (HAPE-S), 7 HAPE-S and 5 controls (C-S) were exposed to simulated altitude of 4000 m for 24 hours in a hypobaric chamber. Minute ventilation and its components (V_T , V_{E} , f_E) as well as the endtidal gases ($P_{ET}O_2$, $P_{ET}CO_2$) were measured in all subjects being in a standardized sitting posture at Zurich level (ZH [450m]) and after 20 min (HA1), 22 hours (HA2) and 24 hours (HA3) at 4000m. At ZH and HA2, the measurements were repeated in a supine and standing posture. In addition, the arterial oxygen saturation (SaO_2) was continuously recorded. At 4000m, V_T was consistently lower in the HAPE-S, and the increase in V_T reached its maximum in the HAPE-S at HA3 ($\Delta V_T = 1.93 \pm 2.77 \text{ ml min}^{-1} \text{ mean} \pm \text{SD}$) and in the C-S at HA4 ($4.52 \pm 4.16 \text{ ml min}^{-1}$), being significant ($p < 0.05$) only in the C-S. During the whole exposure, in the HAPE-S V_T tended to be smaller, and f_E as well as the $P_{ET}CO_2$ tended to be higher, whereas the $P_{ET}O_2$ showed no difference between the groups. Breathing through the mouthpiece during ventilation measurements increased the SaO_2 at 4000m in the HAPE-S, significantly at HA1. This effect was most pronounced in the supine posture, in which the lowest SaO_2 -values in the HAPE-S were found. These data provide evidence, that during the first 24 hours of hypobaric hypoxia HAPE-S exhibit a lower hypoxic ventilatory drive with relative hypoverilation compared to the C-S. The increase in SaO_2 during mouthpiece-breathing might be explained by an altered pattern of breathing in terms of a higher V_T and a lower f_E leading to an enhanced alveolar ventilation. Together with the postural changes in SaO_2 the results also point to a higher ventilatory inhomogeneity in the HAPE-S during the first 24 hours at high altitude.

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RESPONSE OF NITRIC OXIDE PATHWAY TO L-ARGININE INFUSION IN HIGH ALTITUDE HYPOXIA

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Nitric Oxide (NO) is formed in the endothelial cells by the action of a constitutive form of NO synthase. During hypoxic pulmonary hypertension the endothelial L-arginine / NO pathway can be impaired. Moreover, the efficiency of inhaled NO has been demonstrated in high altitude pulmonary edema. To evaluate NO synthesis in acute hypoxia, 7 subjects were infused L-arginine (30 g) during 30 min under normoxia (condition N) and after 24 h at 4,350 m (condition H). Plasma concentration of citrulline (CIT) (measured by chromatography with ninhydrine coloration) was used as an index of NO synthesis. CIT, oxygen saturation (SaO_2), blood pressure (BP), heart rate (HR) and acute mountain sickness score (AMS) were measured at rest and at 15, 30 and 45 min after start of infusion. Hypoxia significantly decreased CIT at 30 and 45 min by 25 % and 27 % respectively ($p < 0.05$). CIT was significantly different from baseline at 30 and 45 min ($p < 0.01$) in N but only at 45 min ($p < 0.05$) in H. L-arginine infusion significantly increased SaO_2 at 30 and 45 min in H ($p < 0.01$ and $p < 0.05$ respectively) and did not affect BP and HR. Subjects who experienced symptoms of AMS showed slightly decreased AMS score with arginine infusion but no significant correlation was found between AMS and CIT. The decrease in CIT may be due to an enhanced turn-over or to a blunting of NO synthesis in acute hypoxia. In this latest hypothesis, impairment of NO release suggests a hypoxia-induced blunting of the constitutive endothelial NO synthase after 24 hours of exposure to high altitude.

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INTERMITTENT HYPOXIA ALTERS HYPOXIC VENTILATORY RESPONSES (HVR)

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BACKGROUND Intermittent hypoxic training (IHT) is used in the Ukraine for prevention and treatment of some diseases and for training of athletes. **HYPOTHESIS** We proposed that IHT by altering neurotransmission in the carotid body enhances HVR. **METHODS** HVR to isocapnic, progressive hypoxic rebreathing, in sitting (SIT) and supine (SUP) positions, lung ventilation and gas exchange while breathing air and during 3 min of breathing 11 % O₂ were studied in 12 healthy young males (age 24.6 ± 1.9 years) (1-IHT) and 6 healthy males (age 24.2 ± 2.3) were sham trained in the same manner as the study group without a decrease in P_{O_2} (S-IHT). All subjects were studied before and after 15 days of IHT which was carried out using the rebreathing technique down to $P_{O_2} = 35$ mm Hg for 6-7 min sessions with 10 min rests 3 times daily for 15 days. **RESULTS** HVR was found to be similar in SIT and SUP at low levels of hypoxic challenge (S) but significantly higher (45%) during severe hypoxia (S₂) (P_{O_2} from 60 - 35 mm Hg). After 15 days of IHT there was a significant increase in HVR in SIT and SUP. S₂-SIT 70% and SUP 100%, S₂-SUP 158% and SUP 200%, maximal lung ventilation: SIT 35% and SUP 78%. After IHT during sustained hypoxia there was enhanced respiratory reactivity evidenced by a 36% increase in lung ventilation and a 22% increase in alveolar ventilation. No significant changes in S-IHT were observed. The greatest responses occurred in T-IHT with hyperactive type of breathing. T-IHT with high and low increases in HVR showed different changes in serum chemiluminescence, lipid peroxidation and enzymes activity. **CONCLUSION** We speculate that reactive oxygen species, which are produced during hypoxia-reoxygenation (IHT), play an important role in neurotransmission in the carotid bodies.

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EFFECTS OF HYPOXIA ON THE GONAD OF MALE OCHOTONA CURZONE AND RAT^a

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Effects of hypoxia on the gonads of male pikas and rats were studied by simulated different altitude (5km and 7km) in the well-ventilated hypobaric pressure chamber for 24h 7d and 20d, comparing with that at 2.3km of altitude. The experiment shows that the plasma estrogen (E₂) level of male pikas increased obviously from 29.7±7.0 pmol/L to 87.43±26.8 pmol/L (at 5 km, $p < 0.05$) and 65.2 ±20.9 pmol/L (at 7km, $p < 0.05$) respectively for exposure of hypoxia 24h; from 47.1±10.3 pmol/L (102.5±29.8 (at 5km, $p < 0.01$) and 124.1±39.6 pmol/L (at 7km, $p < 0.01$) for 7d; The level of plasma testosterone (T) of rats increase from 96.5±27.9 nmol/L to 132.6±32.3 nmol/L (at 5km, $p < 0.05$) and 137.5±33.8 nmol/L (at 7km, $p < 0.001$) for exposed to hypoxia 24h, the effect was much stronger in groups of exposure of hypoxia 7d, the plasma T increase to 142.5±38.8 nmol/L (at 5km, $p < 0.01$) and 181.6±48.5 nmol/L (at 7km, $p < 0.005$) respectively. When rats were exposed to hypoxia for 20d, the level of plasma T decreased sharply from 76.4±18.6 nmol/L to 47.9±11.4 (at 5km, $p < 0.05$) and 42.2±11.7 (at 7km, $p < 0.01$); The ratio of testis weight to body weights in Pikas remain unchanged at simulated 5km of altitude, but decrease from 0.058±0.003 to 0.042±0.003 (, $p < 0.05$) at 7km for exposure of hypoxia 7d, on contrary, the ratio of rats increased from 0.61±0.05g to 0.69±0.02g (at 5km, $p < 0.01$) and to 0.71±0.03g (at 7km, $p < 0.001$). The gaps among the seminiferous tubule of rats obviously enlarged compared with 2.3km group, but the seminiferous tubule of Pikas kept unchanged at all simulated altitude for 7d. Those suggest that E₂ may play a role in an adaptation to hypoxia in Pikas and acute, chronic hypoxia exposure affect the function of male rat gonads.

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EFFECTS OF HYPOXIA ON THE HYPOTHALAMIC GnRH LEVEL OF MALE RAT AND *OCHOTONA CURZONIAE**

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The experiments have been carried out by simulated 5km and 7km of altitude for 24h, 7d and 20d to testify whether hypoxic stress effects on the hypothalamic GnRH and further on reproductive-endocrine function of male rats and *OCHOTONA CURZONIAE* (Pika, a plateau native mammal well-adapted to altitude). Exposed to hypoxia for 24 h, the level of hypothalamic GnRH of both male rats and Pikas decrease obviously from 3.58 ± 0.25 ng/H to 2.86 ± 0.36 ng/H ($p < 0.05$) and from 3.32 ± 0.32 ng/H to 1.72 ± 0.36 ng/H ($p < 0.01$) at 7km of altitude. Exposure of hypoxia for 7d, both at 5km and 7km, the level of hypothalamic GnRH of male rats was without marketable changes, but that of Pikas significantly decreases from 2.92 ± 0.11 ng/H to 2.40 ± 0.52 ng/H ($p < 0.05$) and 1.22 ± 0.16 ng/H ($p < 0.01$) respectively. Exposed to hypoxia for 20d, there was no noticeable changes in the level of hypothalamic GnRH of rats at 5km, but at 7km of altitude, the content of hypothalamic GnRH of rats decrease from 3.14 ± 0.33 ng/H to 2.6 ± 0.17 ng/H ($p < 0.05$), by contrary, the level of hypothalamic GnRH of Pikas increase from 2.75 ± 0.70 ng/H to 4.35 ng/H. In summary, those results indicate that there could be a different adaptive mechanism of hypothalamic GnRH between male rats and Pikas in hypoxia stress and adaptation.

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BIOLOGICAL RESPONSE TO EXOGENOUS TRH IN HUMANS DURING A SHORT STAY AT HIGH ALTITUDE

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The biological response to a combined injection of GRF, GnRH and TRH was studied in 8 healthy men (aged 23-34 yr) both at Bobigny (altitude 50 m; Normoxia : N) and after a 3 to 4 day stay at the Vallois observatory (altitude 4,350 m; Hypoxia : H). Subjects were healthy unacclimated volunteers (no stay above 1,000 m during the 3 preceding months), used to mountain climbing but moderately trained. They were transported by helicopter to the Vallois observatory in less than 15 minutes. Exercise was minimal during both periods and ambient temperature at Vallois observatory was stable around 20°C. Results concerning TRH-responding Hormones (TSH, total and free T₃ / T₄, Prolactin) are presented here. Blood samples were obtained 15 and one minute before injection (basal values) and 15, 30, 45, 60 and 90 minutes after the injection. Serums from both N and H periods were kept frozen in liquid nitrogen until assayed in a same run at BIOMERIEUX laboratories by means of an automated immunoassay (mini VIDAS). Hypoxia induced no change in serum TSH whatever the time of sampling, while total and free thyroid hormones were increased at all times ($p < 0.01$ or better). Prolactin was decreased in H both in basal condition and in response to TRH ($p < 0.05$ and < 0.01 respectively). These results suggest that TRH actions on the release of both prolactin and TSH are dissociated in H. Decreased prolactin may be related to an increase in dopamine, a potent inhibitor of its production (not measured in this study). Our data confirm that exposure to high altitude increases serum concentrations of thyroid hormones independently of pituitary function or with a modification in the set-point for the pituitary negative feedback. Relations between this biological status and a probable hemoconcentration remain to be elucidated before concluding that this state of subtle hyperthyroidism may reflect an adaptative phenomenon to the hypoxic stress.

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INFLUENCE OF HIGH INTENSITY EXERCISE IN NORMOXIA ON LUNG DIFFUSION CAPACITY

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Strenuous exercise in hypoxic environment is a cause of hypoxic pulmonary edema, but it is to be examined the influence of high intensity exercise in normoxia on pulmonary gas exchange. Methods: 17 healthy, male subjects (25 ± 4 yrs, 77.1 ± 6.5 kg, $\dot{V}O_{2\text{max}} 4.1 \pm 0.6 \text{ l min}^{-1}$) performed 60 min of cycling at approx. 86 % of $\dot{V}O_{2\text{max}}$ breathing room air. Cardiac output (\dot{Q}_c), $\dot{V}O_2$ and lung diffusion capacity DLCO were measured by acetylene and CO rebreathing at rest, during exercise, and after the test.

Results: DLCO decreased during the test in 6 of the subjects (D) from 62.1 ± 5.6 to $54.5 \pm 5.6 \text{ mm min}^{-1} \text{ mmHg}^{-1}$ after 40 min of exercise (12.2%, $p = 0.04$), whereas DLCO was constant in the others (59.9 ± 7.4 to $62.1 \pm 11.6 \text{ mm min}^{-1} \text{ mmHg}^{-1}$). At this time, power was unchanged in both groups (change: -1.1 vs -2.6% , ns), but there was a tendency to lower $\dot{V}O_2$ (-9.4% vs $+9.8\%$, ns) and lower \dot{Q}_c (-4.4% vs 2.7% , ns) in D compared to controls. During the last minutes of the test, D decreased power by 9.4%, $\dot{V}O_2$ decreased 11.9%, \dot{Q}_c by 8.9% and DLCO by 16.7%. There were no sign. differences between DLCO at rest and 25 min after the test.

Conclusion: In 6 of 17 healthy subjects, DLCO was decreased significantly after 40 min of constant load high intensive exercise. The decrease in DLCO was not in proportion to smaller changes in $\dot{V}O_2$ or \dot{Q}_c , and these subjects could not maintain power during the remaining test time. These findings indicate that intensive exercise can itself disturb gas exchange in healthy subjects.

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THE RESPONSE TO HIGH ALTITUDE ON THE JAPANESE ALPS : FINDINGS ON A PULSE OXIMETER

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From May 26th to May 30th, 1995, arterial oxygen saturation (SpO_2) and pulse rate (PR) were monitored with pulse oximeter (N-20P, Nellcor) in 11 subjects (5M, 6F, age 19-40yr) to determine the response to high altitude on the Japanese Alps. One Subject developed high-altitude cerebral edema (HACE). This 21-year-old man, who had been trained at a height of 2500m, presented with severe pulsatory headache with objective gait ataxia when he temporarily reached a height of 2750m at 4 days, however he had no deficits because he quickly climbed down. Of the remaining 10 subjects, six were in normal condition and 4 had acute mountain sickness (AMS) which did not require therapy. We analyzed the regression lines of the duration of time at height 2500m on SpO_2/PR ratio (Noguchi's ratio). For both normal and AMS subjects, there was a positive relationship between the variables ($Y = 0.85 \pm 0.09X$, $r = 0.39$ for normal; $Y = 0.77 \pm 0.07X$, $r = 0.53$ for AMS) while the HACE subject showed a line with a negative slope ($Y = 1.01 \pm 0.12X$, $r = 0.83$). We next defined the acclimatization point as when the SpO_2/PR ratio was equal to 1 ($Y = 1$). This point in X was 1.6 days in normal subjects and 3.3 days in AMS subjects, but there was no X point in HACE subject. This study demonstrates that normal and AMS subjects show a gradual increase in the length of stay in SpO_2/PR ratio while acclimatizing in a few days. On the other hand, there is no gradual increase and acclimatization doesn't occur in HACE subject. Many cases need be evaluated in future trials because of a small number of subjects were studied here.

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MYOELECTRIC EVIDENCE OF PERIPHERAL FATIGUE DURING DYNAMIC CONTRACTIONS IN SEVERE NORMOBARIC HYPOXIA: RELATIONSHIPS WITH FIBRE COMPOSITION
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iEMG of the m. vastus lateralis was analyzed during cycle ergometry in male subjects (n=8). Two work trials were conducted, one under normoxia (N), the other under normobaric hypoxia (NH) ($F_2O_2=0.116$), each trial lasting 10 minutes. The absolute power output (180 W) was the same for both trials and was equivalent to 77(±4)% of maximum heart rate in N. Maximal voluntary isometric contractions (MVC) were performed after each trial to assess changes in force, muscle fibre conduction velocity (MFCV), electromechanical delay (EMD), median frequency of EMG (MF) and maximal iEMG (iEMG_{max}). Biopsy samples were obtained from the m. vastus lateralis to determine fibre composition using sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE). At the end of both work trials iEMG was significantly elevated compared with starting values, however iEMG recorded in NH exceeded N values by 15%. At the end of the NH trial myoelectric evidence of 'peripheral' muscle fatigue was observed as reflected by: a decrease in force, MFCV and MF with an increase in EMD and iEMG_{max}/force ratio. iEMG_{max} was unchanged. No differences in any of these variables were observed after the N trial. Mean (SD) lactate concentrations following NH and N trials were 9.2(4.4) mmol¹ and 3.5(1.1) mmol¹, respectively. Results indicated that an increase in motor unit recruitment and rate coding was needed in NH to maintain the required power output. Subjects with higher compositions of type II motor units in the m. vastus lateralis accumulated more lactate ($r=0.80$) and demonstrated greater reductions in MFCV ($r=0.67$) and MF ($r=0.71$) following work in NH.

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REPEATED REOXYGENATION AS THE IMPORTANT FACTOR OF INTERVAL HYPOXIC TRAINING
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Interval hypoxic training (IHT) represents the adaptation to numerous cycles of relatively brief normobaric hypoxic exposures followed by periods of reoxygenation. The duration, intensity and number of such exposures depends on the aim of adaptation and individual characteristics of the organism. Studies of different authors using various models of hypoxia (ischemia) suggest that many events which are supposed to be important factors of hypoxic effects (e.g. lipid peroxidation) occur mainly during the reoxygenation period. We suppose that this period is extremely significant for adaptation and, thus, repeated reoxygenation episodes are critical and very important for IHT. Our results support this concept. Analysis of metabolic-hormonal homeostasis in young healthy volunteers shows that changes of metabolite and hormone levels in blood serum during reoxygenation period often exceed those during normobaric hypoxia (11% O_2 , 10 min) or are quite opposite. Patterns of cross-correlations between different biochemical parameters during reoxygenation are different from those both in basal state and during hypoxia. During reoxygenation, cross-correlations between heart rate, expiratory volume (VE) and brain auditory evoked response (BAER) latency, characteristic for hypoxic period, disappear. Stable cross-correlations between BAER before and BAER after IHT, as well as VE before and VE after IHT revealed during reoxygenation period suggest the important role of the initial reaction of VE and BAER to reoxygenation in the adaptation to interval hypoxia.

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Influence of hypoxia caused by exposure to high altitude on plasma levels of Adrenomedullin (ADM).

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Adrenomedullin (ADM) is a novel potent vasoconstrictor peptide recently isolated from pheochromocytoma tissue by monitoring its elevating activity on platelet cAMP. ADM is known to be involved in chronic pulmonary hypertension. Its role in acute pulmonary hypertension due to hypoxia in humans is not clear. Hypoxia-induced acute pulmonary hypertension at high altitude is well known. We therefore studied the influence of hypoxia caused by exposure to high altitude on plasma ADM levels. METHODS: Fourteen healthy volunteers were investigated at low altitude (470 m) and over four days at high altitude (3560 m). Arterial blood gases were analyzed before taking blood samples for ADM radioimmunoassay. Blood samples were collected in tubes containing EDTA, centrifuged at 1500 g for 10 minutes and the plasma was immediately frozen. Plasma ADM levels were measured by radioimmunoassay after extraction using C-18 SEP cartridges. RESULTS: Plasma ADM concentration was 12.44±3.2 pg/ml at low altitude and increased to 30.24±5.6 pg/ml at high altitude at first day of exposure ($p<0.01$). Over the next days of high altitude exposure plasma ADM concentration decreased slightly, but without significance. All volunteers had normal blood gases at low altitude and P_0 was decreased to 52.3±6.4 mmHg at high altitude. CONCLUSION: This study shows that plasma ADM levels are increased at high altitude and ADM may represent an important factor in the mechanism to counteract pulmonary hypertension in high altitude exposure.

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A ¹H-MRS EVALUATION OF THE CREATINE-PHOSPHOCREATINE POOL IN HUMAN MUSCLE

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Phosphocreatine (PCr) has been shown to effectively buffer ATP levels at high work rates in skeletal muscles. Our main consideration was to assess whether or not the pool of PCr and Cr (C_{tot}) is the same in different metabolic states. 12 healthy power trained(PT) athletes (VO_2 47.6 ± 6.5 ml/kg/min) and 12 healthy endurance trained(ET) athletes (VO_2 65.0±4.9 ml/kg/min) completed a plantar flexion of the right foot against an increasing load until volitional fatigue. This was performed while lying supine in a 3 Tesla superconducting magnet with the medial gastrocnemius centered in a circumscribing coil. Total work production was calculated for the entire activity. Immediately following exhaustion a pressure cuff was inflated for 5 min (>350 mmHg superior to the knee) to allow collection of spectra prior to PCr resynthesis. A PRESS (Point Resolved Spectroscopy) sequence was used to resolve the ¹H-visible Cr/PCr peak during rest and fatigue. Standardized echo time (TE) of 100ms for 164 averages was used in collecting data from a 4.5 cm³ volume of interest (VOI) in the m.gastroc. Upon removal of the cuff recovery of C_{tot} was assessed for 10 min. Following the initial trial, 6 subjects from each group undertook a Cr loading regime for 7 days (5g doses at 4 times per day). The other 12 subjects took sucrose as a placebo. On the final day of loading, subjects returned to repeat the exercise protocol and the same measurements were collected. There was no significant improvement in performance for either subject group, although PT did show a significant increase in NMR visible C_{tot} and had the capacity to utilize this larger Cr store during exercise.

Comparisons of rest vs. ischemic fatigue states in both groups, indicated a 3 fold increase in the ¹H-MRS visible pool of C_{tot} . After 10 min of recovery the pool returned to within 27% of its resting state. These results suggest that there is a separate pool of Cr in the muscle which may be unavailable to creatine phosphokinase until the onset of intense exercise.

ABSTRACTS

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EFFECTS OF HYPOXIC CONDITIONS AT 3550M ON LEVELS OF CORTISOL AND TESTOSTERONE IN SALIVA ¹Tschop M, ²Feldmeier H, ³Toepper M, ⁴Härtmann G, ⁵Fischer R, ⁶Häutmann H, ⁷Kiepl R, ⁸Dressendorfer RA, ⁹Stansburger CJ, ¹⁰Mountain Medicine Study Group and ¹¹Neuroendocrine Unit, Medical Clinic, University Hospital, Ziemssenstr. 1, 80336 Munich, Germany. After free diffusion through the salivary gland epithelial cells, the bioactive fraction of serum cortisol and testosterone can be detected in saliva. Effects of acute hypoxia in high altitude on total steroid hormone serum levels were described earlier, neglecting the influence of reversible inactivation by binding to transport proteins. Salivary cortisol (SC) and salivary testosterone (ST) were measured by in-house time-resolved fluorescence immunoassays (sandwich-biotin system, europium marker, lower detection limits: 0.45 nM, 16.5 nM, respectively); 10 healthy male volunteers were transported from 550m to 3550m, where they stayed for 72 hours. Saliva was collected three times a day (8%, 13%, 18%) from two days before up to two days after the exposure to 3550m. While mean O₂ saturation decreased from 97% (550m) to 88% (3500m), SC showed a significant increase from (mean±SE) 6.68±1.12nM (550m) to 14.29±2.71nM (day 1 at 3500m, $p<0.022$). A following decrease of 33.6% SC from day 1 to day 3 (both at 3550m) was interpreted as a sign for acclimatization and return to normoxia led to baseline levels of SC within 24 hours. ST showed a non significant decrease from (mean±SE) 5749.7±798.2pM/12h (Area under the curve (AUC), at 550m) to 4782.1±848.6pM/12h (AUC, day 1 at 3550m). Saliva samples were taken with 48 h after return to normoxia (4219.7±397.4pM/12h, AUC). The SCST ratio (SC/ST) is proposed to be the most sensitive stress indicator, showing a significant increase from (mean±SE) 0.023±0.004 (550m) to 0.104±0.05 (day 1 at 3500m, $p<0.008$). Both, SC and ST-levels showed a circadian profile with high morning and low evening levels. Measurement of SC and ST could serve as a useful monitoring system of hypoxic stress in men. Stressfree saliva sampling avoids positive bias caused by venipuncture as well as immediate freezing and centrifugation under field conditions. A pronounced effect of hypoxia on SC-levels compared to an only moderate increase of total serum cortisol levels could be due to a decreasing blood pH, elevating the proportion of unbound steroids in serum.

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BIOENERGETICS OF THE TCA CYCLE AND GLYCOLYSIS IN HYPOXIC RAT SKELETAL MUSCLE: MEASUREMENT BY ¹³C MAGNETIC RESONANCE SPECTROSCOPY

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A "reverse" TCA cycle flux from fumarate to succinate in oxygen limited skeletal muscle has been proposed to augment the ATP produced by glycolysis in contracting muscle. We examined this scheme by monitoring the patterns of incorporation of [3-¹³C]lactate and [U-¹³C]acetate into TCA cycle and other metabolic intermediates in hypoxic rat skeletal muscles. We used ¹H and ¹³C magnetic resonance spectroscopy and NADH linked fluorometric assays to assess net pathway flux in resting versus contracting muscle. Inconsistent with the succinate dehydrogenase-linked scheme, contraction caused no decrease in [aspartate] or [α -ketoglutarate] and no increase in [alanine] or [succinate] in either gastrocnemius or soleus muscle, but did cause a decrease in [[3-¹³C]alanine]/[[3-¹³C]lactate] and [[3-¹³C]glutamine] and an increase in [[2,3-¹³C]succinate]/[[4-¹³C]glutamate] in gastrocnemius muscle. Taken together, our data are inconsistent with the idea that hypoxic rat skeletal muscle generates usable metabolic energy via a fumarate to succinate flux. In contrast, our model of energy production is linked to succinyl-CoA synthetase, with both forward and reverse TCA cycle flux, the malate-aspartate shuttle, pyruvate cycling and glycolysis.

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WOMEN AT ALTITUDE: THE EFFECT OF ALTITUDE AND THE MENSTRUAL CYCLE ON SYMPATHETIC AND PARASYMPATHETIC INPUTS TO THE HEART

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This study was designed to examine the effects of altitude and menstrual cycle on sympathetic and parasympathetic nervous system (SNS and PNS) sympathetic input to the heart using spectral analysis of heart rate variability (HRV). Nine women (ages 19-34 years) with normal menstrual cycles (>28 days) were studied at sea level during each phase of their menstrual cycle as well as during the early (days 3-4) and late (days 10-11) periods of acclimatization to high altitude on the summit of Pikes Peak, CO (elev. 4300m). At altitude, each woman was studied in either the follicular (F) or luteal (L) phase of the menstrual cycle. ECG measurements were obtained under conditions of quiet rest and during periods of controlled breathing at 6 bpm and 12 bpm. At sea level, total spectral power increased in the F phase relative to the L phase, suggesting increased autonomic input to the heart during the F phase. SNS activity appeared to rise during early exposure to high altitude, as indicated by increased ratio of low frequency HRV (0.04-0.15 Hz) to high frequency HRV (0.15-0.30 Hz), while total SNS activity (SNS activity = 1 - SNS ratio as the ratio of high frequency HRV to total HRV) were reduced. All changes were similar in the L and F groups. At days 10-11, SNS activity declined to levels intermediate between sea level and days 3-4 values and total variance was increased compared with days 3-4. It appears that women, like men, undergo SNS activation and PNS withdrawal during short term exposure to high altitude and that these changes occur independent of menstrual cycle phase.

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WOMEN AT ALTITUDE: ELEVATION IN SYSTEMIC ARTERIAL PRESSURE OVER TIME AT 4,300 M

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Prior studies performed in men at 4,300 m have shown elevations in resting, ambulatory, and exercise systemic arterial pressures in response to chronic hypoxia linked to heightened sympathetic stimulation. To address whether a similar response occurs in women we studied 16 young women (ages 19-34 yrs), with normal menstrual cycles, during each phase of the menstrual cycle at sea level and at 4,300 m, high altitude. 24 hour ambulatory blood pressure recordings were obtained at sea level, early (days 3-4) and later (days 10-11) during a period of residence at this altitude. In addition, invasive arterial recordings were obtained at rest and during submaximal exercise at sea level and after 10 days at 4,300 m. At sea level, there was no significant effect of menstrual cycle phase on ambulatory resting blood pressure values. Daytime mean arterial pressure (MAP) rose from 85±1 to 94±1 mmHg after 2-4 days at 4,300 m, with no further rise after 10 days. The increase in MAP was related to increases in both systolic and diastolic blood pressure in response to prolonged hypoxia. Resting intraarterial MAP was also greater after 10 days at 4,300 m compared to sea level (90±2 vs 80±2 mmHg), although there were no differences noted during submaximal exercise. There did not appear to be a significant effect of menstrual cycle phase on the elevations in MAP seen at high altitude. Thus, systemic arterial pressure appears to rise over time at high altitude in women, similar to that reported in men. Sympathetic activation is suspected to play a major role in this chronic altitude pressor response.

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EFFECT OF GENDER ON THERMOREGULATION AND SURVIVAL OF HYPOXIC RATS

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Hypothermia is a documented response to hypoxia but little is known about possible gender differences. Since female rats have a greater hypoxic ventilatory response than males, we hypothesized that females would be more tolerant of hypoxia. We studied 18 female and 18 male Long-Evans rats. Radiotelemetry transmitters for body temperature (T_b) were implanted under general anesthesia (Nembutal®). Rats were exposed to 21, 16, 12, 10, 8, 6, 4, 2% O₂ (balance N₂) for 30 min each in chambers kept at 31° (clamped) or 20° C (hypothermic). Survival was defined as ataxic and unresponsive. Females were more hypoxia tolerant than males, often enduring 2% inspired O₂ (~13 km altitude). This was correlated with a lower T_b in the hypothermic group but not in the clamped group. Hypothermia increased "survival" of rats independent of gender. When T_b was clamped, females rats showed significantly greater survival than males. Thus, separate mechanisms (hypothermia or ventilation) may be acting to increase tolerance of clamped and hypothermic female rats to severe hypoxia.

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EFFECT OF β -ENDORPHIN ON CORTICOTROPIN-RELEASING FACTOR INDUCED BY SIMULATED HYPOXIA*

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In the present study we examined the effects of β -endorphin (β -EP) on CRF contents in median eminence (ME) and hypothalamus (Hy) of rats and Tibet Plateau native mammals (*Ochotona curzoniae*) during acute hypoxia. Experimental animals were exposed to 7000m altitude for two hours, CRF contents in ME and Hy significantly reduced to 65.33% and 59.7% of control group (2300m). At 7000m altitude, CRF contents in ME and Hy of rats increased significantly after administration of β -EP icv 0.01, 0.1, 1 nM. Naloxone administration (10 nM) reversed partly the effects of β -EP (0.1 nM) in rat. β -EP had no significant effect on CRF release in *Ochotona curzoniae*. These results suggest that β -EP may inhibit rat CRF release from ME during acute hypoxia.

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THE CIRCADIAN RHYTHM OF HYPOTHALAMIC ACH AND NE IN BRAIN IN *MICROTUS OECONOMUS**

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The root voles (*Microtus oeconomus*) kept in nature light, there were circadian rhythms in levels of hypothalamic Ach and NE in brain. The highest level-the peak of hypothalamic Ach content was 5.26±0.13 μg/mgW.W at about 1:00 A.M and the lowest was 2.67±0.17 μg/mgW.W at about 20:00 P.M. On the contrary, the highest level-the peak of NE content in brain was 518.4±82.6 ng/gW.W at about 10:00 A.M the lowest was 325.5±69.0 ng/gW.W at about 1:00 A.M.

In hypobaric chamber, these root voles were exposed at simulated altitude of 5 and 7 Km for 2h, the Ach content in the hypothalamus and NE content in brain presented a significant decreased levels. At the hypoxic exposure for 24h, the Ach content of hypoxia at 5km groups and the NE content showed a continued decrease but the Ach content of hypoxia at 7km groups kept the same level as hypoxia groups of 2h.

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THE EFFECT OF HYPOXIA ON DEVELOPMENT OF HYPOTHALAMIC CORTICOTROPIN-RELEASING FACTOR FOR NEONATAL RATS*

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It was studied in present paper that the development of hypothalamic corticotropin-releasing factor (CRF) of neonatal rats after birth, age of 5-35 days rats exposed to simulated altitude hypoxia in hypobaric chamber. When the animals were exposed to 5km altitude for 10, 15, 20, 25 and 30 days, their body weight increased with age but more slowly than control group (2.3km). The hypothalamic CRF was also developed with age and still lower than same-age control, especially on 30 and 35 days (exposed for 25 and 30 days), just 64.8% and 57.9% of the control group. Meanwhile, the pituitary cAMP was higher but not significantly than control when exposed for 20 and 25 days, and decreased rapidly for 30 days, only 20.8% of the control group. It was also found that pregnant rats could not complete their pregnancy, thus, their fetuses could not develop well at 7km altitude. The CRF of 20-day-old fetus (exposed for 17 days) was higher than control group at 5km altitude. It was suggested that the development of hypothalamic CRF was markedly inhibited when neonatal rats exposed to 5km altitude for 25 and 30 days, perhaps this was the key stage of development and the hypoxia could not provide enough oxygen for their metabolism.

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THE DIAGNOSIS OF ACUTE MOUNTAIN SICKNESS IN PRE-VERBAL CHILDREN

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The Lake Louise Scoring System (LLSS) defines acute mountain sickness (AMS) in adults but cannot be applied to pre-verbal children. The objective of this study was to establish the diagnostic criteria for AMS in pre-verbal children. Children ≥ 3 and ≤ 36 months old from the Denver, CO area (1609 m, 5280 ft) without known cardiopulmonary disease or acute illness were studied over 4 separate days. A fussiness score (FS) was used as the headache equivalent score. The remainder of the LLSS was modified into a pediatric symptom score (PSS) assessing appetite, vomiting, playfulness and ability to nap. We defined the children's LLSS (CLLS). FS+PSS. Parents recorded the FS at 1100, 1300, 1500 & 1700 hours and PSS at 1500 hours of each study day. Days 1 & 2 were measurements at home, day 3 reflected travel without altitude change to Ft. Collins, CO (1615 m, 1615 ft), and day 4 involved travel to the Keystone, CO summit lodge (3488 m, 11,444 ft). On days 3 & 4 pulse oximetry (SpO₂), pulse (P), and respiratory rate (RR) were measured, and adults completed the LLSS. Twenty-three subjects (14 boys), ages 20.7 ± 9 mos. participated. The mean CLLS demonstrated no differences on days 1-3 if FS ≥ 4 and PSS ≥ 3 . On day 4 five subjects (21.7%) had AMS (defined as a CLLS ≥ 7) and these scores normalized 2 hours after descent. No differences existed between the subjects with or without AMS regarding SpO₂, P and RR. Forty-five adults participated and 9 (20%) had AMS by the LLSS after 4 hours at altitude. We define AMS in pre-verbal children as a CLLS ≥ 7 with FS ≥ 4 and PSS ≥ 3 , in the setting of recent altitude gain. The incidence of AMS in pre-verbal children (21.7%) was similar to adults (20%).

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THE MECHANISM OF CALCIUM ACTION ON BRAIN INJURY AND THE PROTECTIVE EFFECT OF GANGLIOSIDES DURING HYPOXIA

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In order to explore the mechanism of Ca²⁺ action on brain injury and the protective effect of gangliosides during hypoxia, the changes in free intracellular Ca²⁺, active-calcmodulin (CaM), Ca²⁺/CaM-dependent protein Kinase II (CaM Kinase II) activity, tubulin and ultrastructure in cerebral cortices of rats were studied during acute hypoxia (at a simulated altitude 7000m, for 5 hrs). The sixty Wistar rats weighing 180-220g were randomly divided into normoxic group (N), acute hypoxia group (A1) and acute hypoxia group (A2, injecting gangliosides). As compared with N, Ca²⁺ increased by 17.3% in A1 (P<0.01) and 136.6% in A2 (P<0.01), CaM decreased by 13.2% in A1 (P<0.05) and by 4.1% in A2, CaM Kinase II activity decreased by 7.8% in A1 (P<0.05) and by 4.0% in A2, tubulin decreased by 31.7% in A1 (P<0.01) and 13.8% in A2 (P<0.01), respectively. However, Ca²⁺ in A1 increased and tubulin in A2 decreased more significantly than those in A2. It was found that the synapses (synapses/100 μ m²) decreased by 14 in A1 and 11 in A2, the synaptic vesicles (vesicles/synapse) decreased by 16 in A1 and 10 in A2, and the intact mitochondria decreased by 25.4% in A1 and by 20.0% in A2. These results indicate that the dramatic increase of free intracellular Ca²⁺ can lead to a significant reduction in the content of active-CaM, activity of CaM Kinase II and content of tubulin which participate in the process of synaptic degeneration and cause brain injury during acute hypoxia, and the gangliosides play an important role in protecting brain from injury via stabilizing free intracellular Ca²⁺, CaM, CaM Kinase II and tubulin under the same condition.

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PITUITARY-ADRENAL RESPONSIVENESS TO EXERCISE IN MAN AND WOMAN AFTER CHRONIC HIGH ALTITUDE EXPOSURE

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The hypothalamus-pituitary-adrenal system is known as the most important mediator of neuroendocrine response to stress and it is strongly stimulated by maximal exercise. Since high altitude (HA) exposure also represents an environmental stress condition, in a group of twelve climbers (9 males, mean age 32.9 ± 1.0 , and 3 females, mean age 35.7 ± 3.9) we studied the pituitary-adrenal response to an exhaustive treadmill graded exercise (Bruce protocol, modified), before and within ten days after a fifteen-day stay at an altitude of at least 4400 m. Blood samples for plasma ACTH, Cortisol, β -endorphin and lactate were collected at rest, at exhaustion and in the post-exercise recovery phase (1, 3, 5, 10, 15, 20 and 30 min). Maximal O₂ consumption (VO_{2max}) and heart rate (HR) were also measured. After chronic HA exposure VO_{2max} decreased about 11% (P<0.01) while HR only slightly decreased; moreover, basal ACTH, β -endorphin and lactate levels were not different, while Cortisol significantly decreased (18.2 \pm 2.8 pre, vs 13.2 \pm 1.9 post, p<.02). After HA, ACTH, β -endorphin and lactate, but not Cortisol responses to exercise were significantly reduced. The same curve profile was observed both in men and in women, but the differences of ACTH profiles between pre and post HA exposure were significantly more pronounced in women (p<.001). In conclusion, HA exposure impairs the ACTH and β -endorphin response to exercise. This phenomenon, more evident in women than in men, is not conditioned by basal Cortisol levels and it could be due in part to the different lactate response to exhaustive exercise after HA. Moreover, chronic HA exposure may give evidence of a gender-linked responsiveness of the hypothalamus-pituitary-adrenal system.